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A Comprehensive Analysis of SGLT2-inhibition in Type 2 Diabetes and Heart Failure

Singh, Jagdeep

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A COMPREHENSIVE ANALYSIS OF SGLT2-INHIBITION IN TYPE 2 DIABETES AND HEART FAILURE

Jagdeep Singh Surmukh Singh
MBBS (Manipal), MRCP (UK)

Degree of Doctor of Medicine

University of Dundee

2019

DEDICATION:

I dedicate this work to the three people who will read it in its entirety; the internal examiner, the external examiner and the research fellow that succeeds me.

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INDEX OF ABBREVIATIONS

6MWT	Six-minute walk test
ACCORD	Action to Control Cardiovascular Risk in Diabetes
ACE-I	Angiotensin converting enzyme inhibitor
ACR	Albumin : creatinine ratio
ACS	Acute coronary syndrome
ADA	American Diabetes Association
ADHERE	the Acute Decompensated Heart Failure National Registry
ADVANCE	Blood Pressure and Glucose Lowering for the Prevention of Vascular Disease in High Risk Patients with Type 2 Diabetes
AE	Adverse events
ARB	Angiotensin II receptor blocker
ATP	Adenosine triphosphate
BB	Beta-blockers
BCA	Body composition analysis
BHB	Beta-hydroxy butyrate
BIOSTAT	Systems Biology Study to Tailored Treatment in Chronic Heart Failure
BMI	Body mass index
BNP	B-type natriuretic peptide
BP	Blood pressure
CANVAS	Canagliflozin Cardiovascular Assessment Study
CHARM	Candesartan in Heart Failure-Assessment of Reduction in Mortality and Morbidity Program
CI	Confidence interval
CKD	Chronic kidney disease
CMR	Cardiac magnetic resonance imaging

CONSORT	Consolidated Standards of Reporting Trials
CPET	Cardio-pulmonary exercise test
CrCl	Creatinine clearance
CRF	Case report forms
CV	Cardiovascular
CVD	Cardiovascular disease
DAG	Diacylglycerol
DAPA-HF	Study to Evaluate the Effect of Dapagliflozin On the Incidence of Worsening Heart Failure or Cardiovascular Death in Patients with Chronic Heart Failure
DBP	Diastolic blood pressure
DCCT	Diabetes control and complications trial
DECLARE-TIMI 58	Multicentre Trial to Evaluate the Effect of Dapagliflozin on the Incidence of Cardiovascular Events
DEXA	Dual-Energy X-ray Absorptiometry
DM	Diabetes mellitus
DPP-IV	Dipeptidyl peptidase-IV
EASD	European Association for the study of Diabetes
ECG	Electrocardiograph
ECM	Extracellular matrix
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
EMPA	Empagliflozin in Heart Failure: Diuretic and Cardio-Renal Effects
EMPA-HEART	Effects of Empagliflozin on Cardiac Structure in Patients with Type 2 Diabetes
EMPA-REG OUTCOME	Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients

EMPEROR-Preserved	Empagliflozin Outcome Trial in Patients with Chronic Heart Failure with Preserved Ejection Fraction
EMPEROR-Reduced	Empagliflozin Outcome Trial in Patients with Chronic Heart Failure with Reduced Ejection Fraction
EPO	Erythropoietin
EVEREST	Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan
EXAMINE	Cardiovascular Outcomes Study of Alogliptin in Patients with Type 2 Diabetes and Acute Coronary Syndrome
FFA	Free fatty acid
FPG	Fasting plasma glucose
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GLP-1	Glucagon like peptide-1
GLUT	Glucose transporter
GP	General practitioner
Hb	Haemoglobin
HbA1c	Haemoglobin A1c (Glycated haemoglobin)
Hct	Haematocrit
HDL	High density lipoprotein
HF	Heart failure
HFmrEF	Heart failure with mid-range ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFrfEF	Heart failure with reduced ejection fraction
HR	Hazard ratio
hsCRP	High sensitivity C-reactive protein
IFG	Impaired fasting glucose

IGT	Impaired glucose tolerance
IHD	Ischaemic heart disease
IL	Interleukin
IQR	Interquartile range
IU	International units
JVP	Jugular venous pulse
KM	Kaplan–Meier
LA	Left atrium
LDL	Low density lipoprotein
LV	Left ventricular
LVAD	Left ventricular assist device
LVEDV	Left ventricular end diastolic volume
LVESV	Left ventricular end systolic volume
LVSD	Left ventricular systolic dysfunction
MACE	Major adverse cardiovascular events
MAP	Mean arterial pressure
MCS	Mental Component Summary
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and healthcare products regulatory agency
MI	Myocardial infarction
MLHF	Minnesota Living with Heart Failure
MRA	Mineralocorticoid receptor antagonist
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NGSP Program	National Glycohemoglobin Standardization

NHS	National Health Service
NO	Nitric oxide
NT-proBNP	Amino-terminal pro B-type natriuretic peptide
NYHA	New York Heart Association
OD	Once daily dosing
OGTT	Oral glucose tolerance test
OPTIMISE	Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure
Oxi-LDL	Oxidized low density lipoprotein
PAD	Peripheral arterial disease
PAI-1	Plasminogen activator inhibitor-1
PCS	Physical Component Summary
PCT	Proximal convoluted tubules
PET	Positron emission tomography
PG	Plasma glucose
PGI ₂	Prostacyclin I-2
PND	Paroxysmal nocturnal dyspnoea
PPAR- α	Peroxisome proliferator-activator alpha
PRESERVED-HF	Dapagliflozin in Preserved Ejection Fraction Heart Failure
QOL	Quality of life
RAAS	Renin-angiotensin-aldosterone system
RECEDE-CHF	SGLT2 Inhibition in Combination with Diuretics in Heart Failure
REFORM	Research into the effect of SGLT2-inhibitor on left ventricular remodelling in patients with heart Failure and diabetes mellitus
ROS	Reactive oxygen species

SAVOR TIMI-53	Does Saxagliptin Reduce the Risk of Cardiovascular Events When Used Alone or Added to Other Diabetes Medications
SBP	Systolic blood pressure
SD	Standard deviation
SF-36	36-item Short form survey
SGLT	Sodium-glucose linked cotransporter
SHARE	The Scottish Health Research Register
SOLVD	Studies of Left Ventricular Dysfunction
SPCRN	Scottish Primary Care Research Network
STEMI	ST-Elevation myocardial infarction
SU	Sulfonylurea
T2DM	Type 2 diabetes mellitus
TECOS	Sitagliptin Cardiovascular Outcomes Study
TG	Triacylglycerol
TNF	Tumour necrosis factor
TrueFISP	True fast imaging with steady state precision
TZD	Thiazolidinediones
UK	United Kingdom
US-FDA	United States Food and Drug Administration
UTI	Urinary tract infection
VADT	Veterans Affairs Diabetes Trial
VCO ₂	Carbon dioxide consumption rate
VE	Minute ventilation
VO ₂	Oxygen consumption rate
WHO	World Health Organisation
WRF	Worsening renal function

ACKNOWLEDGEMENTS

This work would not have been possible without the help and guidance from a number of people, but above all I wish to give praise and thanks to God for blessing me with a beautiful life.

I wish to also express my deepest gratitude to the following individuals who have been instrumental to my work:

Professor Chim Lang, for not only giving me an opportunity of a lifetime to pursue this research, but also for taking a chance on this unknown person half a world away. Also, to Professor Allan Struthers for his wisdom and enlightening corridor discussions.

Ify Mordi for spending hours in a darkened room looking at grey and black images of hearts and for his insights into analysing the dataset. Also to Christopher Gingles, Patrick Liu, Mohapradeep Mohan, Sunny Jabbal, Victor Chong, Daniel Levin, Alex Brown, Bayan Soujeri, Arvind Manoharan, Fatima Baig, Lynn Douglas and Isobel Ovens for being excellent colleagues and ready sources of help in times of need.

A particularly special thank you goes out to Amir Fathi and Keeran Vickneson who were eager medical students at the time but made significant contributions to the tedious but vital work required for the success of the trial.

The staff at the Tayside Clinical Trials Unit, Immunoassay Biomarker Core Laboratory, The Scottish Health Research Register for their excellent support.

All the patients for giving their time and energy, in spite of illness, to participate in the REFORM trial.

To my mentor and Guru, Professor Dato' Dr. Suren Menon for always challenging me and elevating me to ever greater heights. You have unlocked my true potential and shown me that the sky *isn't* the limit.

And finally, to my family for their patience and sacrifice. To my three mothers Harvinder Kaur, Harjit Kaur and Satwinder Kaur (in order of appearance) my debt to you is insurmountable. To my darling baby Kyreena, daddy is sorry he has not spent time playing with you, that will change now. To Jessy, my life partner, thank you for putting up with my intermittent grumpiness and all those lost weekends and holidays – although I now have one less excuse, I suspect nothing much will change there. I love you.

DECLARATION

I hereby declare that I am the sole author of this thesis. All the data were collected and analysed by myself, apart from cardiac magnetic resonance imaging analysis which was performed by Dr. Ify Mordi. The data collection and preliminary analysis were conducted during my appointment as the European Foundation for the Study of Diabetes Clinical Research Fellow at the Division of Molecular and Clinical Medicine, University of Dundee between January 2015 and December 2016.

All the work described in this thesis is original and has not been published in its entirety elsewhere at the time of print. All the references cited herein have been consulted by myself. This work has not been previously submitted for consideration of a higher degree.

Signed:

Date: 23 July 2019

THESIS SUMMARY

Type 2 diabetes mellitus (T2DM) and heart failure (HF) are diseases that commonly co-exist and have been shown to increase mortality. There is a variety of treatment options available to treat T2DM, however in the context of HF, many have been shown to not have any beneficial effects on reducing morbidity or mortality, while some are demonstrably harmful.

The sodium-glucose linked co-transporter type 2 (SGLT2)-inhibitor class of drug was developed as a novel anti-diabetic agent that acts independent of the insulin-incretin pathway to lower blood sugar. Part of the off-target effects that have been seen with this drug class include weight loss, blood pressure reduction and diuresis – all of which are key in reducing cardiovascular risk, particularly in HF.

An unexpected but consistent finding in large cardiovascular outcome trials (which are now mandatory for all new anti-diabetic agents) across the entire SGLT2-inhibitor class was that of improved HF outcomes. The mechanism of this effect was poorly characterised and required further exploration.

The Research into the Effect of SGLT2-inhibitor on Left Ventricular Remodelling in Patients with Heart Failure and Diabetes Mellitus (REFORM) trial, addresses the literature gap in this area, and is the first to study the effects of SGLT2-inhibition specifically in the HF population. It showed no difference between groups in the primary endpoints of left ventricular (LV) end diastolic volume or LV end systolic volume; +4.15 ml; 95%CI: -18.52 to 26.83; p=0.714 and +0.96 ml; 95%CI: -17.07 to 19.00; p=0.915 respectively. Patients on

dapagliflozin had weight reduction; -1.97kg; 95%CI: -3.99 to 0.05; p=0.056 lower diastolic blood pressure; -6.58mmHg; 95%CI: -11.93 to -1.23; p=0.017 higher haemoglobin; +1.16 g/dL; 95%CI: 0.60 to 1.74; p<0.001 and increased serum beta-hydroxybutyrate; +0.04 mmol/L; 95%CI: 0.001 to 0.08; p=0.045. They were also more likely to stop or reduce their dose of loop diuretics; 50.0% vs 8.7%; p=0.005. Further exploratory analysis revealed that dapagliflozin may improve measures of LV remodelling in patients with baseline LV ejection fraction of 45% or more.

We have demonstrated, for the first time, that the effects of dapagliflozin seen previously in the T2DM population remains consistent in the HF population with T2DM. We have also have generated new avenues of research to improve our understanding of this drug class and its potential future use.

1. INTRODUCTION

1.1 TYPE 2 DIABETES; A GROWING EPIDEMIC

1.1.1 Defining and diagnosing diabetes

The World Health Organisation (WHO) has published guidelines for the diagnosis and classification of diabetes since 1965. In its most recent iteration in 2006, the WHO defines diabetes as “*a condition primarily defined by the level of hyperglycaemia giving rise to microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life.*”(2) The most current diagnostic criteria for diagnosing diabetes is summarised in Table 1.

FPG \geq 126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*
OR
2-h PG \geq 200 mg/dL (11.1 mmol/L) during OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water.*
OR
A1C \geq 6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose \geq 200 mg/dL (11.1 mmol/L).
*In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

Table 1. Diagnostic criteria for diabetes mellitus(3)

FPG=Fasting plasma glucose; PG=Plasma glucose; OGTT=Oral glucose tolerance test; WHO=World Health Organisation; DCCT=Diabetes control and complications trial; NGSP= National Glycohemoglobin Standardization Program

There are four broad types of diabetes, namely Type 1 diabetes (due to autoimmune pancreatic beta cell destruction leading to absolute insulin deficiency) , Type 2 diabetes (due to progressive loss in pancreatic beta cell function, on the background of insulin resistance), Gestational Diabetes (diabetes diagnosed in the second or third trimester of pregnancy in an individual not previously known to have diabetes) and specific types of diabetes due to secondary causes (such as neonatal diabetes, maturity-onset diabetes of the young, disease of the exocrine pancreas [such as cystic fibrosis and pancreatitis] and drug / chemical induced diabetes [such as glucocorticoid use, treatment of HIV/AIDS or after organ transplantation])(3)

The class of diabetes considered in this work is limited to that of Type 2 diabetes mellitus (T2DM) only.

1.1.2 Incidence and prevalence of Type 2 diabetes

In 2016 the WHO published its Global Report on Diabetes which showed the global prevalence of all types of diabetes in 2014 was 8.5%, representing 422 million individuals. This report also revealed diabetes was directly responsible for 1.5 million deaths in 2012, with a further 2.2 million deaths due to cardiovascular disease, chronic kidney disease and other conditions related to higher than normal blood glucose levels.(4) T2DM is by far the commonest type of diabetes and a recent study analysing published data from 45 countries representing nearly 90% of the world's population estimates that in 2018 there are 500 million prevalent cases of T2DM. They project the greatest growth in T2DM prevalence will be in low-income countries over the next 10 years, which will greatly increase the burden on already strained health services in those countries.(5)

Diabetes-UK states that diabetes is the fastest growing healthcare threat the United Kingdom is facing. In its facts and stats update 2019, it estimates 4.7 million people have diabetes in the UK, including 1 million who are unaware of the disease as they remain undiagnosed.(6) The 2016 Scottish Diabetes Survey revealed there are nearly 300,000 individuals with diabetes in Scotland (prevalence 5.4%), with 88.3% of them having T2DM. The incidence of T2DM in 2016 was 316 per 100,000 population per year.(7)

1.1.3 Type 2 diabetes and cardiovascular disease

There is a well-recognised link between T2DM and cardiovascular disease (CVD). The pathophysiology that triggers the development of CVD starts well before a patient develops frank T2DM. Prediabetes is a term used for individuals whose glycaemic level does not fulfil criteria for diagnosing diabetes but is too high to be considered normal. Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are two clinical conditions by which prediabetes manifests itself.(3) The diagnostic criteria for prediabetes is summarised in Table 2.

FPG 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) (IFG)
OR
2-h PG during 75-g OGTT 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L) (IGT)
OR
A1C 5.7–6.4% (39–47 mmol/mol)
*For all three tests, risk is continuous, extending below the lower limit of the range and becoming disproportionately greater at the higher end of the range.

Table 2. Diagnostic criteria for prediabetes(3)

FPG=Fasting plasma glucose; IFG=Impaired fasting glucose; OGTT=Oral glucose tolerance test; IGT=Impaired glucose tolerance

In the past, the cut-off for diagnosing diabetes was thought to represent the threshold of risk for developing microvascular pathology and thus CVD. We now recognise that no such threshold exists, and that CV risk is more of a spectrum, becoming disproportionately greater the higher the degree of hyperglycaemia.(3,8) Insulin resistance is the hallmark of prediabetes and underpins many of the pathophysiological processes associated with it.

Additionally, prediabetes is strongly associated with central obesity, hypertension, hypertriglyceridemia and or low HDL; the so-called metabolic syndrome, which is itself associated with increased microvascular and macrovascular risk.(9)

Subclinical inflammation has been strongly associated with prediabetes and insulin resistance, as both the inflammatory and insulin signalling pathways are very closely linked to each other.(10) Elevated levels of high sensitivity-C reactive protein, interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)- α have been shown in individuals with prediabetes, especially those who are at high risk of developing T2DM.(11) These cytokines, in turn, inhibit phosphatidylinositol 3-kinase dependent signalling of nitric oxide (NO) synthesis. Additionally, insulin resistance and hyperglycaemia results in increased free fatty acid (FFA), reactive oxygen species (ROS) and endothelin-1.(12,13) This combination of impaired NO synthesis, elevated ROS and increased levels of the potent vasoconstrictor endothelin-1 results in endothelial dysfunction. Indeed, endothelial dysfunction is the earliest manifestation of glycaemia-related vascular disease.(14)

Insulin resistance is also associated with several changes in lipid and lipoprotein levels. As an individual with prediabetes progresses into frank T2DM, elevated very low density lipoprotein (VLDL)-triglycerides and reduced high density lipoprotein (HDL)-cholesterol (and by extension, apolipoprotein A1 which is the major apolipoprotein in HDL) is commonly seen.(15,16) Importantly, HDL and apolipoprotein-A1 are responsible for the removal of excess cholesterol from monocyte-derived macrophages (cholesterol ester-

engorged macrophages are also known as foam cells).(17) Although total cholesterol and low density lipoprotein (LDL) are frequently normal in patients with T2DM, there are compositional changes in the LDL by way of increased small dense LDL particles (which are the result of increased VLDL-triglycerides) and an increase in the absolute number of LDL particles. Small dense LDL particles readily enter the arterial wall, cause increased synthesis of procoagulants and are more easily oxidised than larger particles.(14) This, along with the pro-inflammatory state discussed above accelerates atherosclerosis, which is one of the key pathological changes in cardiovascular disease.

Insulin resistance and T2DM increases thrombotic risk via multiple mechanisms. Insulin resistance is associated with elevated levels of plasminogen activator inhibitor-1 (PAI-1) which suppresses fibrinolysis.(18) Numerous studies have also linked the pathophysiologic process of T2DM (e.g. pro-inflammatory state, altered lipoprotein profile) with increased coagulation factors VII, VIII, XII and fibrinogen.(19) T2DM also affects platelet function. Insulin antagonises the action of adenosine diphosphate, platelet activating factor and collagen on platelet cell surface receptors, thereby preventing platelet activation and aggregation. Although hyperinsulinemia is a feature of T2DM (and one could therefore expect to see an anti-platelet effect), numerous studies have shown platelet aggregation is actually upregulated in the context of insulin resistance, suggesting that the effect of insulin resistance supersedes that of hyperinsulinemia in this regard.(18,20-22) Finally, healthy endothelial cells produce anti-aggregants such as NO and prostacyclin I-2 (PGI₂). The endothelial dysfunction resulting from insulin resistance and T2DM

suppresses the production of these molecules and tips the balance toward platelet aggregation and thrombosis.(19)

It has long been argued that patients with T2DM develop HF as a result of diabetes induced-cardiovascular disease or a complication from it such as hypertension or a myocardial infarction. Although that may certainly be a contributing factor, there has been a recent recognition that diabetes itself, by neuro-hormonal, metabolic, inflammatory, microvascular and bio-energetic pathways, may be directly responsible for HF – this will be explored in more detail in section 1.3.2.

1.2 THE GROWING BURDEN OF HEART FAILURE

1.2.1 Defining and diagnosing heart failure

The 2016 European Society of Cardiology Guidelines for the diagnosis and treatment of acute and chronic heart failure defines HF as a “*clinical syndrome characterized by typical symptoms that may be accompanied by signs caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and / or elevated intracardiac pressures at rest or during stress.*”(23) Heart failure is primarily a clinical diagnosis that is supported by investigative findings to prognosticate the disease, identify other co-morbidities and to determine the underlying aetiology, which in some cases may be reversible.

The key in diagnosing HF is the presence of typical symptoms of breathlessness, fatigue, oedema, orthopnoea and paroxysmal nocturnal dyspnoea (PND) which are frequently associated with evidence of an elevated jugular venous pulse (JVP), third heart sound, crackles in the lungs and peripheral oedema.

An important, albeit subjective, clinical measure of determining the symptom burden of HF is the NYHA functional classification. The NYHA classification was first described in 1928, then developed further in 1964 into the 4-numerical-class system used till today in clinical practice and cardiovascular research. To address the subjective nature of the NYHA classification (a limitation recognised by the 1964 Criteria Committee when they described the system as “*only approximate, for it is derived largely by inference from the*

history, by observation of the patient in certain forms of physical activity, and occasionally by direct or indirect measurement of cardiac function in response to standardized exercises. It represents an expression of [the provider's] opinion...”), a further ‘objective assessment’ criteria (classes A to D) was added to the system in 1994.(24) This additional criterion uses measures such as electrocardiogram (ECG), x-rays, echocardiograms and other radiologic assessments to classify the severity of cardiac structural and functional abnormality from ‘no objective evidence of cardiovascular disease’ (Class A) to ‘objective evidence of severe cardiovascular disease’ (Class D). Therefore, a complete evaluation should include the patient’s functional capacity (Class I to IV) and objective assessment (A to D). (Table 3) Although using both criteria increases the reliability of the NYHA classification, the objective assessment component is rarely quoted in routine clinical practice.(25)

Functional Capacity	Objective Assessment
Class I. Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	A. No objective evidence of cardiovascular disease.
Class II. Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	B. Objective evidence of minimal cardiovascular disease.
Class III. Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	C. Objective evidence of moderately severe cardiovascular disease.
Class IV. Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	D. Objective evidence of severe cardiovascular disease.

Table 3. NYHA Classification of Heart Failure

B-type natriuretic peptide (BNP) and the amino terminal portion of its precursor, proBNP (NT-proBNP) are cardiac biomarkers that have now been widely accepted as tools to assist in the workup for HF. BNP is released predominantly in the ventricles in response to myocardial stretch as a result of increased end-diastolic pressure and/or volume.(26,27) It plays a prominent role in the compensatory response to HF by way of increased natriuresis, diuresis, vasodilation as well as inhibiting the sympathetic nervous system (by reduced catecholamine secretion) and the renin-angiotensin-aldosterone system (RAAS) (by reduced renin secretion).(28) Patients with normal concentrations of BNP / NT-proBNP are unlikely to have HF. Cut-offs of 35 pg/mL for BNP and 125 pg/mL for NT-proBNP in the chronic setting and 100 pg/mL and 300 pg/mL respectively in the acute setting have a negative predictive value between 0.94 and 0.98.(29-31) It is important to note that these biomarkers have much lower positive predictive values, and therefore their main use should be in ruling out HF rather than establishing the diagnosis.(23) There has been interest in using BNP / NT-proBNP to objectively track patients' response to therapy but the data have been mixed; there is evidence to suggest that it correlates to improvements in clinical and objective markers of congestion (such as jugular venous pulsation, orthopnoea, weight, inferior vena caval diameter and pulmonary capillary wedge pressure) (32) but not to improvements in hard outcomes such as hospitalisation for HF or CV mortality.(33)

As discussed above, another important aspect of assessing heart failure includes imaging the heart to determine the degree of functional impairment as well as to identify structural abnormalities that may be responsible for, or

the result of, the heart failure. Echocardiography, which is an inexpensive, accurate and accessible tool, is the mainstay in this regard. Advancements in echocardiography technology and technique now mean that detailed assessments of systolic and diastolic function can be made, and overall ventricular function (measured as ejection fraction [EF]) can be measured to a high degree of reliability. More recently this has led to further subclassifying heart failure, according to degree of LV dysfunction, to heart failure with reduced ejection fraction (HFrEF), heart failure with mid-range ejection fraction (HFmrEF) and heart failure with preserved ejection fraction (HFpEF) with echocardiographic LVEF cut-offs of <40%, 40-49% and \geq 50% respectively.(23) This is an important distinction because patients in these different categories (particularly in the case of HFrEF vs HFpEF) have different aetiologies, co-morbidities, prognosis and response to therapy.(23,34)

An important but frequently overlooked part of the diagnostic workup for HF is establishing the underlying aetiology. This is important because it may reveal reversible aetiologies such as cardiotoxicity from alcohol / drugs, comorbidities like severe anaemia, thyroid disease, valvular heart disease or even nutritional deficiencies. In some cases, these investigations may also reveal a heritable disease such as familial dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy which will have implications to the patient's family members. (Table 4)

DISEASED MYOCARDIUM		
Ischaemic heart disease	Myocardial scar	
	Myocardial stunning/hibernation	
	Epicardial coronary artery disease	
	Abnormal coronary microcirculation	
	Endothelial dysfunction	
Toxic damage	Recreational substance abuse	Alcohol, cocaine, amphetamine, anabolic steroids.
	Heavy metals	Copper, iron, lead, cobalt.
	Medications	Cytostatic drugs (e.g. anthracyclines), immunomodulating drugs (e.g. interferons monoclonal antibodies such as trastuzumab, cetuximab), antidepressant drugs, antiarrhythmics, non-steroidal anti-inflammatory drugs, anaesthetics.
	Radiation	
Immune-mediated and inflammatory damage	Related to infection	Bacteria, spirochaetes, fungi, protozoa, parasites (Chagas disease), rickettsiae, viruses (HIV/AIDS).
	Not related to infection	Lymphocytic/giant cell myocarditis, autoimmune diseases (e.g. Graves' disease, rheumatoid arthritis, connective tissue disorders, mainly systemic lupus erythematosus), hypersensitivity and eosinophilic myocarditis (Churg–Strauss).
Infiltration	Related to malignancy	Direct infiltrations and metastases.
	Not related to malignancy	Amyloidosis, sarcoidosis, haemochromatosis (iron), glycogen storage diseases (e.g. Pompe disease), lysosomal storage diseases (e.g. Fabry disease).
Metabolic derangements	Hormonal	Thyroid diseases, parathyroid diseases, acromegaly, GH deficiency, hypercortisolaemia, Conn's disease, Addison disease, diabetes, metabolic syndrome, pheochromocytoma, pathologies related to pregnancy and peripartum.
	Nutritional	Deficiencies in thiamine, L-carnitine, selenium, iron, phosphates, calcium, complex malnutrition (e.g. malignancy, AIDS, anorexia nervosa), obesity.
Genetic abnormalities	Diverse forms	HCM, DCM, LV non-compaction, ARVC, restrictive cardiomyopathy (for details see respective expert documents), muscular dystrophies and laminopathies.
ABNORMAL LOADING CONDITIONS		
Hypertension		
Valve and myocardium structural defects	Acquired	Mitral, aortic, tricuspid and pulmonary valve diseases.
	Congenital	Atrial and ventricular septum defects and others (for details see a respective expert document).
Pericardial and endomyocardial pathologies	Pericardial	Constrictive pericarditis Pericardial effusion
	Endomyocardial	HES, EMF, endocardial fibroelastosis.
High output states		Severe anaemia, sepsis, thyrotoxicosis, Paget's disease, arteriovenous fistula, pregnancy.
Volume overload		Renal failure, iatrogenic fluid overload.
ARRHYTHMIAS		
Tachyarrhythmias		Atrial, ventricular arrhythmias.
Bradyarrhythmias		Sinus node dysfunctions, conduction disorders.

Table 4. Aetiologies of heart failure(23)

ARVC=Arrhythmogenic right ventricular cardiomyopathy; DCM=Dilated cardiomyopathy; EMF=Endomyocardial fibrosis; GH=Growth hormone; HCM=Hypertrophic cardiomyopathy; HES=Hypereosinophilic syndrome; HIV/AIDS=Human immunodeficiency virus/Acquired immune deficiency syndrome

1.2.2 Incidence and prevalence of heart failure

HF has been identified as a growing global epidemic with approximately 26 million people with the disease worldwide in 2014.(35) The incidence of HF is difficult to quantify due to variations in the diagnostic criteria and method of detection. Inconsistencies in hospital coding, including 'upcoding' for financial incentive and an inability to distinguish between first and subsequent admissions have been blamed. Additionally, the increasing use of outpatient care for HF mean inpatient data (which forms the bulk of epidemiological data in HF) is unable to capture the true burden of incidence.(36) As a consequence, reported incidence range from 1.0 to 26.0 per 1000 person-years depending on age, gender, ethnicity and geographical location, with the highest incidence seen in elderly males of Afro-Caribbean decent.(36)

In developed nations the prevalence of HF is approximately 1-2% of the adult population. This rises to more than 10% in individuals above the age of 70.(23,37) It is also the commonest cause of hospitalisation in patients above the age of 65.(38) In the UK, more than 500,000 people have HF and the burden of treating this disease is large; accounting for 1-2% of the NHS's overall budget with up to 70% of that cost related to hospitalisation alone.(39) A similar picture is seen in the United States with HF accounting for 1 million hospitalisations annually with an average duration of hospitalisation between 5 to 7 days depending whether it is a primary or secondary (related to co-morbid disease) presentation respectively.(40)

1.2.3 Pathophysiology of heart failure

The pathophysiology behind HF varies depending on the underlying aetiology, or in some cases, aetiologies. When one recognises that HF is a syndrome rather than a disease in and of itself, it is easy to understand how (and why) multiple mechanisms are at play in causing HF. Some examples of these mechanisms (or models) include the haemodynamic, cardio-renal, neurohormonal, abnormal calcium cycling and cell death models.

The haemodynamic model is well-known and using the Frank-Starling principal, it provides a mechanical understanding of the effects of excessive increases in intraventricular end-diastolic volume and stretch on cardiac contractility. In 1967 Eugene Braunwald and colleagues demonstrated decreasing intrinsic contractility of the myocardium with increasing haemodynamic load using isolated cardiac muscles from cats with heart failure.⁽⁴¹⁾ The extracellular matrix (ECM) is an important determinant of the ventricular architecture, and by extension, its function. Remodelling of the ECM from an insult like a myocardial infarction results in fibrosis and thinning of the ventricular wall resulting in altered ventricular loading dynamics. Additionally, the loss of contractile tissue also contributes directly to poorer inotropy. (See cell death model below)⁽⁴²⁾ Conversely, excessive ECM synthesis stimulated by uncontrolled hypertension and/or persistent activation of the RAAS (and resultant aldosterone excess) result in ventricular hypertrophy causing impaired relaxation (ventricular filling) and contraction (ventricular emptying). These changes result in HF albeit with a preserved EF; the so-called HFpEF.⁽⁴³⁾

The kidneys play an integral role in the HF syndrome. The cardio-renal model describes the kidney's role in sodium and fluid homeostasis which is directly related to congestion and the manifest symptoms of HF. Equally, renal function is also dependent on cardiac function; reduced renal blood flow to the glomerulus (and even renal ischaemia in severe cases) from impaired forward flows and peri-renal congestion from increased back pressure are seen in HF. These changes in renal haemodynamics have been established as the primary driving mechanisms of renal impairment in patients with HF.(44,45) (Figure 1)

A further interplay between these two organs relate to the effects of anti-HF medications such as diuretics and RAAS-blockers that frequently strain renal function.

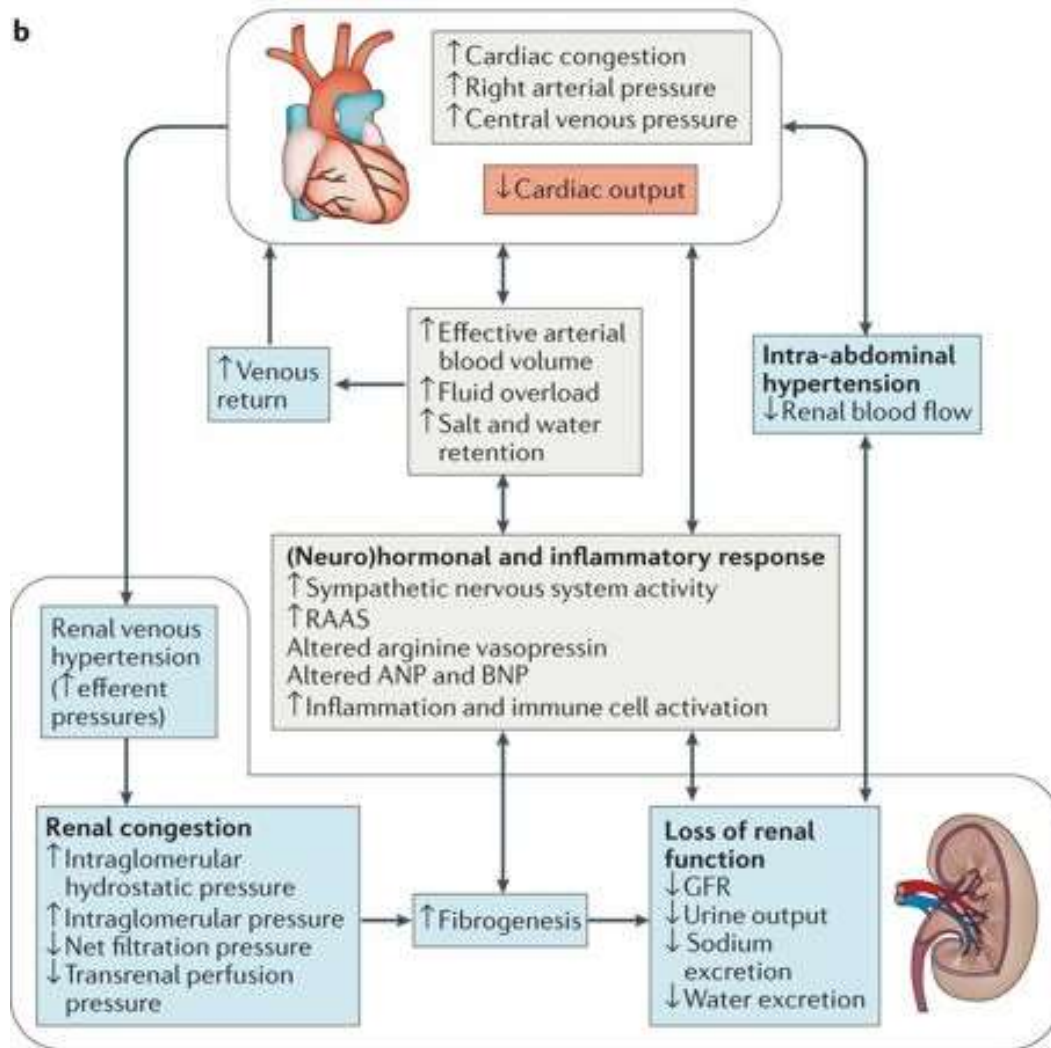


Figure 1. Cardio-renal model of heart failure(45)

RAAS=Renin-angiotensin-aldosterone system; ANP=Atrial natriuretic peptide; BNP=B-type natriuretic peptide; GFR=Glomerular filtration rate

The neurohormonal system is an important determinant of cardiac performance. Seminal work, once again, by Professor Braunwald and colleagues performed in the mid-20th century established the role of the sympathetic nervous system in augmenting cardiac performance during

exercise and states of increased demand.(46) In the acute setting, the sympathetic nervous system and the RAAS increases cardiac contractility, induces vasoconstriction and anti-natriuresis resulting in improved BP and perfusion of vital organs, thereby compensating for the LV dysfunction.(47) However, sustained activation of the neurohormonal system results in maladaptive remodelling of the ventricles and myocardial injury causing further impairment of LV function, thus setting up the vicious cycle that is the hallmark of the neurohormonal model of HF.(42) (Figure 2)

A common feature of all the pathophysiological models discussed above is the compensatory mechanisms to a failing heart results in a spiral of adverse remodelling within the myocardium which then perpetuates the diseased state. Deranged myocardial calcium homeostasis is yet another maladaptive response to ventricular hypertrophy, increased LV end diastolic pressure and sustained RAAS activation.(48) Intracytoplasmic calcium in the cardiac myocyte plays a key role in myocardial contractility as well as in maintaining the electrical stability of the heart.(49) Dysregulation of calcium flux due to dysfunctional ryanodine receptors (RyR2) and the sarcoendoplasmic reticular adenosine triphosphate-driven calcium (SERCA2a) pump alters intracytoplasmic and sarcoendoplasmic calcium concentration and results in weaker myocardial contractility(50) and ventricular tachyarrhythmias. (51,52)

All HF syndromes are associated with increased cardiac myocyte death.(53) As discussed above, this may be the result of abnormal loading conditions, excessive adrenergic activity, prolonged RAAS activation and remodelling, deranged calcium handling or even other factors such as direct cellular

damage from radiation, infiltration (amyloid, sarcoid, glycogen or lysosomal storage disorders), and toxins such as alcohol or chemotherapeutic agents. In other scenarios, autophagy which is a physiological and protective process of intracellular organelle recycling, may be maladaptively upregulated in instances of increased pressure loading leading to unrestrained cell death.(54) Whereas in the elderly, autophagic activity declines resulting in intracellular accumulation of protein aggregates and lipofuscin. These by-products of cellular function lead to increased oxidative stress, impaired cellular metabolism and eventually cell death.(55)

Apart from these classical (generic) mechanisms, there are other more aetiology-specific mechanisms such as the genetic model relating to various inherited cardiomyopathies, the immune model relating to auto-immune and infective cardiomyopathies and the diabetic model which will be discussed in more detail in the next chapter.

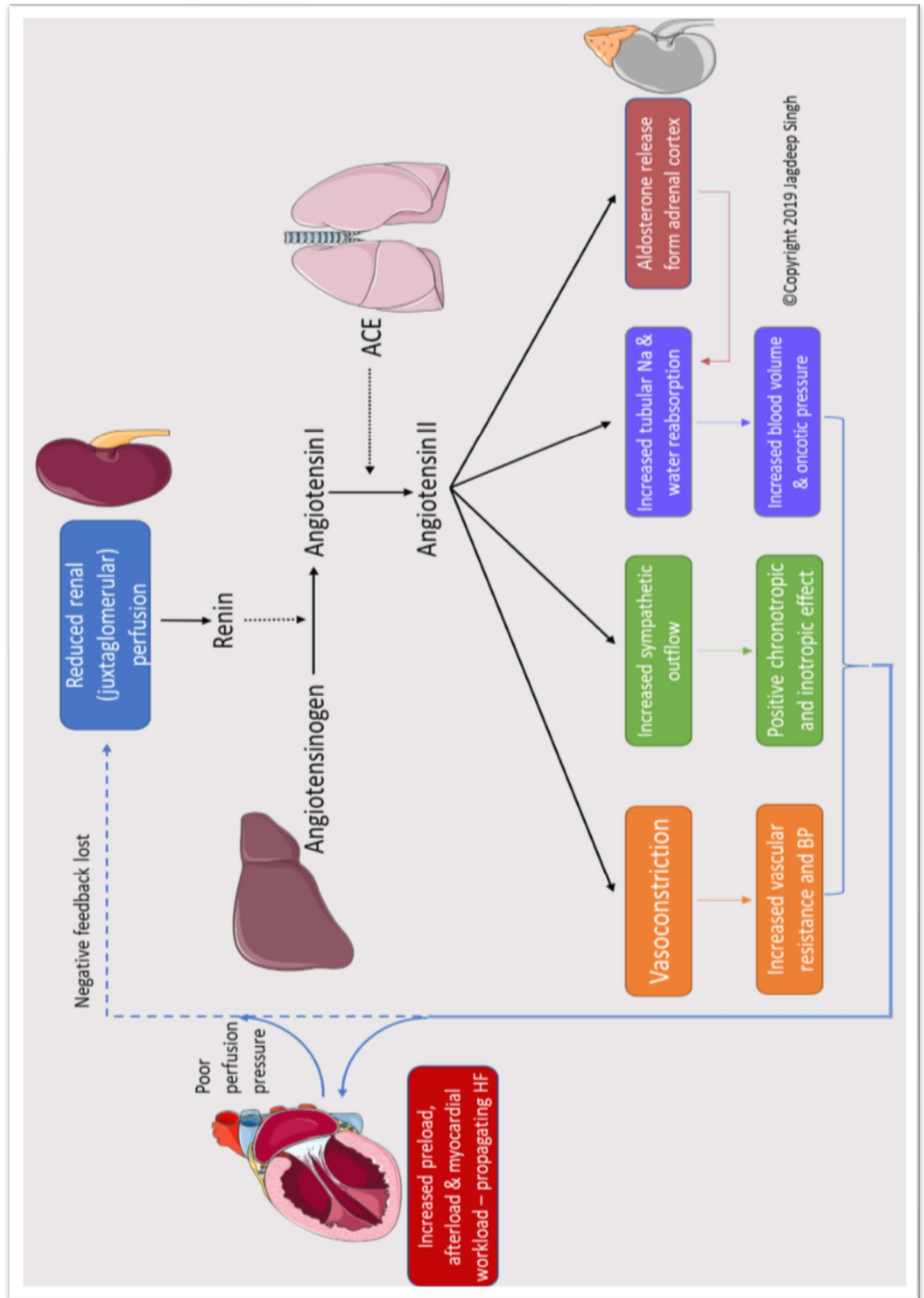


Figure 2. Loss of RAAS negative feedback in heart failure.

1.3 HEART FAILURE AND DIABETES; A LETHAL COMBINATION

1.3.1 The diabetic heart

As discussed in the preceding chapters, the prevalence of T2DM and HF continue to increase. Worryingly, these two potentially lethal conditions in their own right also commonly co-exist; up to 45% of patients with chronic HF have concomitant T2DM.⁽⁵⁶⁾ In a population-based study of individuals 65 years or older, the 5 year survival of a diabetic patient with a new diagnosis of HF was 12.5% compared to over 80% if they remained free of HF. The same study also showed males and Caucasians fared worse than females and Blacks.⁽⁵⁷⁾

Registry data seem to suggest there is no difference in the outcomes of patients with or without T2DM presenting with acute HF. The Acute Decompensated Heart Failure National Registry (ADHERE) showed no difference in in-hospital mortality between both groups, while the Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure (OPTIMIZE-HF) registry did show slightly longer hospitalisations (5.9 vs 5.5 days; $p < 0.0001$), but no mortality difference up to 90 days post discharge (note only 10% of patients were followed up to 90 days).

However in a large, contemporary, prospective analysis of this cohort, Sarma et al looked at data from the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial and found patients with T2DM had higher combined endpoints of CV mortality or HF hospitalisation (HR 1.17; 95% CI 1.04–1.31) for a median of 9.9 months

following discharge for an acute presentation of HF with low ejection fraction, compared to those who did not have T2DM.(58) (Figure 3) These data are more in keeping with the long term, population-based studies that clearly showed a stark difference in mortality when T2DM and HF co-exist.

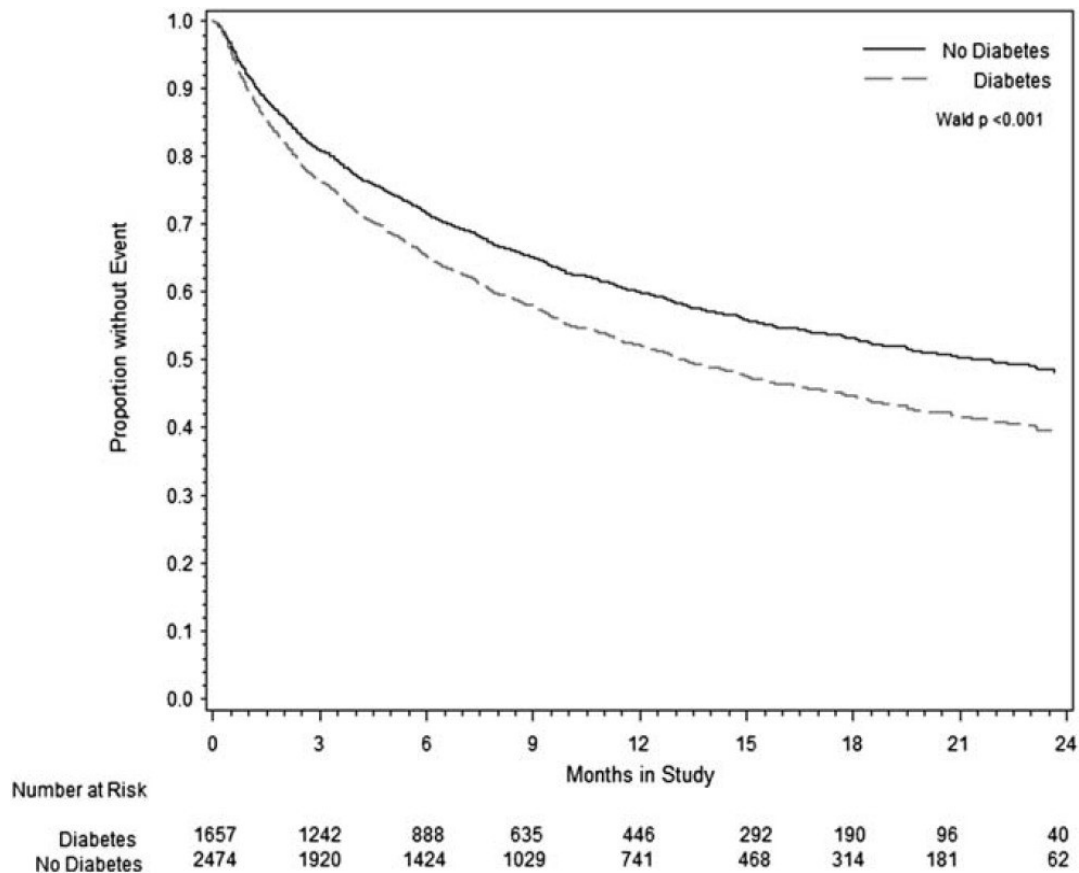


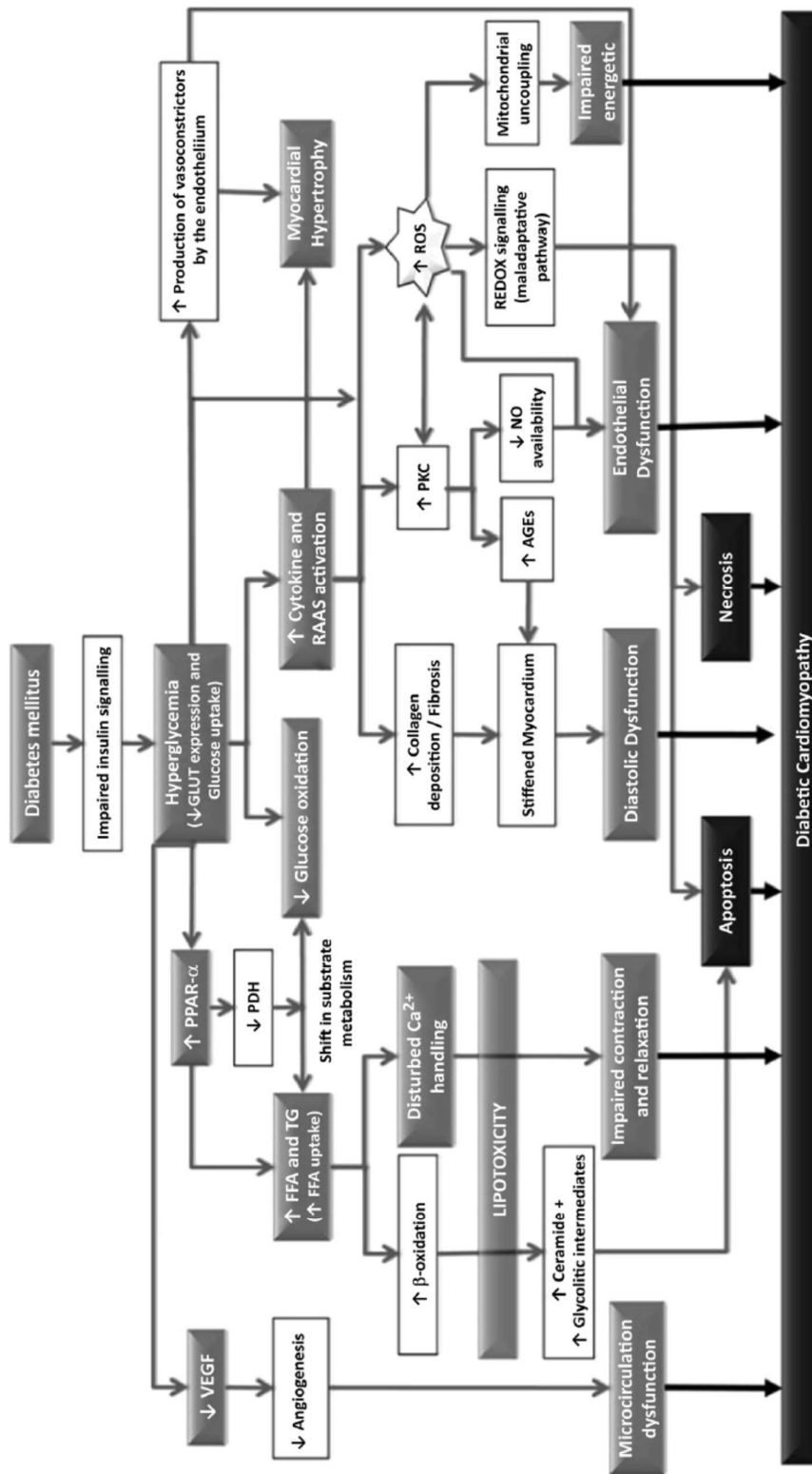
Figure 3. Long term outcomes of patients with or without T2DM following HF hospitalisation(58)

Interestingly there is a bidirectional risk for T2DM and HF. Patients with T2DM have been shown to have an 8% increase risk of HF for every 1% rise in HbA1c.(59) Similarly, patients with chronic HF have markedly increased insulin resistance, which increases their risk for developing T2DM compared

to healthy individuals or even those with coronary artery disease.(60) In the following sections we will explore how these two conditions interact and form a lethal combination.

1.3.2 Diabetes causing heart failure

Following on from the observation of increased mortality and morbidity when T2DM and HF co-exist, it is important to understand the pathophysiology driving this increased risk in patients. Diabetic cardiomyopathy is now becoming more recognised as a pathophysiological entity of its own. Until recently, most authors believed that heart failure was the long-term manifestation of accelerated CV disease due to diabetes, however with a clearer understanding of the molecular pathways that are affected by T2DM, we now recognise how the metabolic, inflammatory, microvascular and neuro-hormonal changes caused by diabetes can result in de-novo cardiomyopathy; the so-called diabetic cardiomyopathy. (Figure 4)



Multiple pathophysiologic mechanisms are activated and modulated by the presence of diabetes mellitus, resulting in abnormalities in the myocardial substrate unique to patients with diabetes mellitus and heart failure. Adapted with permission from Falcao-Pires I, Leite-Moreira AF. Heart Fail Rev 2012;17:325-44. AGE = advanced glycation end product; FFA = free fatty acid; GLUT = glucose transporter facilitators; PDH = pyruvate dehydrogenase; PKC = protein kinase C; PPAR = peroxisome proliferator-activated receptor; RAAS = renin-angiotensin-aldosterone system; REDOX = oxidation and reduction; ROS = reactive oxygen species; TG = triglyceride; VEGF = vascular endothelial growth factor.

Figure 4. Pathophysiology of Diabetic Cardiomyopathy(61)

One of the earliest metabolic changes seen is the switch of myocardial fuel balance away from glucose toward FFA. In the healthy heart, cardiomyocytes are able to freely switch between glucose, FFA, lactate, ketones and other fuel substrates depending on fuel availability, workload and tissue perfusion.(62) In a state of rest, the healthy heart predominantly uses FFA as a fuel substrate because of its dense energy content. However, during periods of increased workload, glucose is preferred as it is a far more oxygen-efficient fuel; although it may liberate less energy per molecule compared to FFA, it has a better energy yield per oxygen atom consumed. (Table 5)

Substrate	P/O ratio***	Energy liberated, kcal/mol of 2-carbon units
Glucose	2.58	223.6
Pyruvate	2.50*	185.7*
Palmitate	2.33**	298**
BHOB	2.50*	243.6*

*Although the difference in energy produced by BHOB (per oxygen atom consumed) is comparable to pyruvate, more energy is derived from BHOB vs. pyruvate due to BHOB being more reduced. If pyruvate were burned in a bomb calorimeter, it would liberate 185.7 kcal/mol of 2-carbon units, whereas the combustion of BHOB would liberate 31% more calories (243.6 calories/2-carbon unit). **Combustion of palmitate generates 298 kcal/mol of 2-carbon units, but there is loss of ATP due to uncoupling proteins generating heat instead of ATP. ***P/O ratio reflects the number of molecules of ATP produced per atom of oxygen reduced by the mitochondrial electron transport chain.

Table 5. Fuel energetics of various substrates(62)

ATP-Adenosine triphosphate; BHOB-Beta-hydroxybutyrate; P/O-Phosphate-oxygen

Similarly, under hypoxic conditions such as myocardial ischaemia or in the failing myocardium, there is an adaptive and cardio-protective response that favours glucose as the primary fuel substrate via enhanced glucose uptake, activation of glycolytic pathway enzymes and reduction of FFA

oxidation.(61,62) By switching the fuel substrate, the heart is able to reduce the supply-demand mismatch and improve myocardial contractile efficiency by increasing the energy production-to-oxygen consumption ratio.

In patients with insulin resistance and T2DM, this ability to switch to a predominantly glucose fuel substrate is lost. Insulin resistance reduces the efficiency of the GLUT-4 glucose transporter protein which is responsible for glucose uptake into the cell.(63) Additionally, there is increased delivery of FFA as a result of enhanced lipolysis and peripheral insulin resistance.(64) Longstanding elevation in intra-myocyte FFA levels activate the nuclear receptor peroxisome proliferator-activated receptor-alpha (PPAR- α), which in turn results in increased mitochondrial FFA transport and oxidation; thereby fixing the myocyte into a FFA-based fuel substrate. FFAs also impair the intracellular insulin signalling pathways, consolidating the insulin resistance of the myocyte and relegating glycolytic pathways even further. Indeed in a recent small study (n=18) using positron emission tomography to measure myocardial FFA consumption with 16-[^{18}F] fluoro-4-thia-palmitate (FTP) in patients with or without T2DM, Mather et al. demonstrated increased myocardial FFA oxidation in patients with T2DM and a failure to fully suppress myocardial FFA oxidation in hyperinsulinemic-euglycaemic clamp. This reflected the persistently elevated rates of myocardial FFA transport contributed by activation of PPAR- α . They also observed reduced myocardial work efficiency in the T2DM group, which they postulated was the result of greater oxygen consumption associated with FFA oxidation.(65)

Apart from predisposing to an adverse myocardial energetic profile, lipotoxicity is another potential factor contributing to diabetic cardiomyopathy. When myocardial FFA uptake outpaces its β -oxidation capacity, the excess FFA is converted to triacylglycerol (TG) leading to cardiac steatosis. Additionally, there is also accumulation of potentially toxic by-products of β -oxidation such as diacylglycerol (DAG) and ceramide.(66) This intra-myocyte build-up of TG, DAG and ceramide generates ROS. Early changes of ROS damage are seen in the sarcoplasmic (endoplasmic) reticulum, where there is inactivation of its calcium-ATPase.(67) This results in reduced sequestration of calcium in the sarcoplasmic reticulum and a cytosolic calcium overload, which in turn causes myocardial fibrosis, hypertrophy and diastolic dysfunction.(66) Further accumulation of ROS induces mitochondrial uncoupling (dysfunction) and eventually apoptosis.(61)

Other non-metabolic factors contributing to the pathophysiology of diabetic cardiomyopathy include microvascular dysfunction, neurohormonal activation and autonomic dysfunction. Advanced glycated end-products are a well described vascular sequela of T2DM resulting in microvascular remodelling, impaired nitric oxide production and endothelial dysfunction. The consequent myocardial ischaemia contribute to myocyte necrosis, fibrosis and hypertrophy.(61) Insulin resistance and T2DM have also been implicated with sympathetic activation as well as upregulation of the RAAS which are deleterious to the heart due to increased cardiac fibrosis, apoptosis, vascular inflammation and oxidative damage.(68,69)

1.3.3 Heart failure causing diabetes

The risk of cardiovascular disease due to T2DM is well recognised but the risk of developing T2DM from established HF is less well studied. Recent work, however, has demonstrated increasing risk of insulin resistance with worsening New York Heart Association (NYHA) functional class in non-diabetic patients with HF(70) as well as increased incidence of T2DM with worsening NYHA functional class in post myocardial infarction (MI) and elderly HF patients.(71,72)

The exact mechanisms behind this observation are still being elucidated, but there are numerous hypotheses that suggest neurohormonal, haemodynamic or even iatrogenic causes to this.

HF induces a maladaptive increase in sympathetic outflow and RAAS activation. Sympathetic activation has been shown to reduce insulin-mediated glucose uptake into skeletal muscles by 25% (peripheral insulin resistance) and increase lipolysis resulting in elevated FFA levels. Increased FFA levels worsen insulin resistance by impairing intracellular insulin signalling pathways and increasing hepatic gluconeogenesis, further worsening hyperglycaemia. Additionally catecholamines also inhibit pancreatic insulin secretion and stimulate hepatic gluconeogenesis and glycogenolysis compounding the hyperglycaemic state.(73) RAAS activation has been implicated with the development of diabetes by way of angiotensin II and aldosterone-induced insulin resistance due to increased oxidative stress and altered intracellular insulin signalling. Aldosterone has also been shown to reduce insulin secretion from isolated pancreatic islets and cultured beta-cells while angiotensin II has

been implicated with pancreatic beta-cell apoptosis.(74) Additionally, subgroup analysis of RAAS-modulating anti-HF therapy such as enalapril in the Studies of Left Ventricular Dysfunction (SOLVD) trial and candesartan in the Candesartan in Heart Failure-Assessment of Reduction in Mortality and Morbidity Program (CHARM) trial showed lower risk of developing T2DM in the treatment arms; HR 0.22 (0.10–0.46, $P<0.0001$) and HR 0.78 (0.64–0.96, $P=0.02$) respectively.(75,76) These findings lend credence to the theory that RAAS activation is involved in the development of T2DM.

The haemodynamic hypothesis proposes impaired perfusion and increased congestion of the pancreas and liver resulting in deranged glucose homeostasis. A study involving 50 patients from a single centre who underwent left ventricular assist device (LVAD) implantation showed significant and sustained reduction in HbA1c up to 1-year post implant.(77) Although not definitive and open to confounding, this study supports the haemodynamic hypothesis. Another potential mechanism is that of skeletal muscle atrophy and the resultant reduced physical activity associated with HF may predispose to a more sedentary lifestyle and, indirectly, to insulin resistance and T2DM.

As more drug classes were licenced for use with HF, there was increasing interest in the potential side-effect profile of these agents, especially around developing T2DM. There was conflicting evidence on this, compounded by significant heterogeneity in meta-analyses comparing the effects of different drug classes. A network meta-analysis (which is able to overcome the issue of heterogeneity due to differing treatment strategies and indirect comparisons of

multiple agents) of various anti-hypertensive agents involving more than 140,000 patients from 22 clinical trials (only 1 of them on patients with HF) indeed did show a protective effect of ACE-i and ARB as well as an increased risk of incident T2DM in patients using diuretics and beta-blockers.(78) In the case of beta-blockers potential mechanisms include direct inhibitory effects on pancreatic beta-cell insulin release and increased peripheral insulin resistance, while patients on diuretics may develop hypokalemia causing reduced insulin secretion.(79)

This bidirectional relationship of T2DM and HF requires the treating physician to always be vigilant of potential early signs of disease progression, if not already presenting with both co-morbidities together. It highlights the importance of understanding their intertwined pathophysiology (Figure 5) and underscores the urgent need for specific, targeted therapy for this unique group of patients.

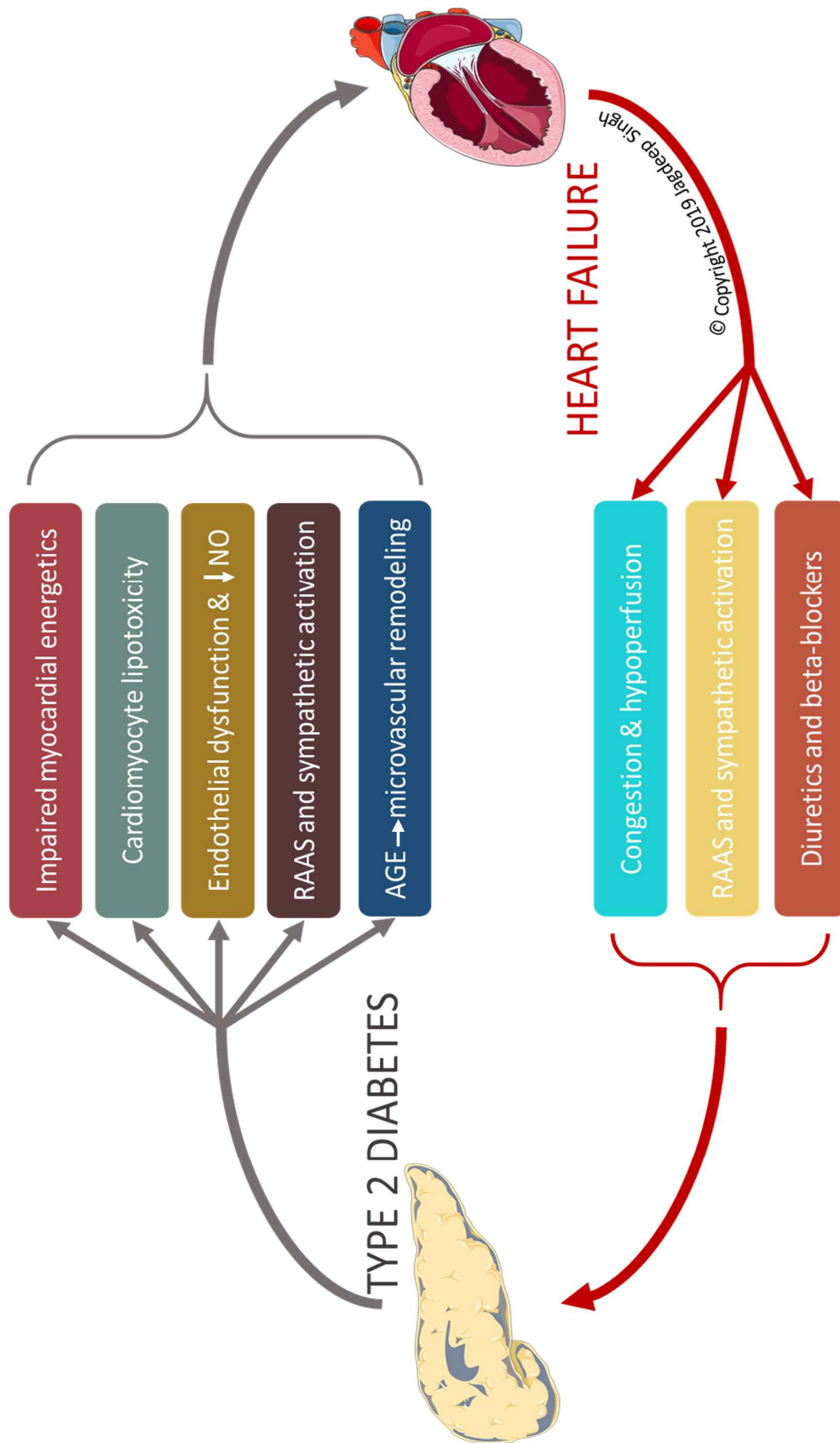


Figure 5. Bidirectional relationship between Type 2 diabetes and heart failure

NO=Nitric oxide; downward arrow= Reduced; AGE=Advanced glycated end-products; sideways arrow=resulting in; RAAS=Renin angiotensin-aldosterone system.

1.4 COMPLEXITIES OF TREATING T2DM AND HEART FAILURE

1.4.1 Overview

The last 3 decades have been a boon for managing T2DM, with rapid development of multiple anti-diabetes medications acting via various mechanisms. Although they all have proven efficacy in lowering fasting blood glucose and HbA1c, their impact on hard outcomes such as reducing CV risk (particularly macrovascular risk) and mortality have been less robust.(80-82)

Of particular concern is their effect on patients with concomitant HF; in some instances, drug classes have a neutral effect on HF-related outcomes, while others may actually increase the risk of incident HF and CV mortality (discussed further in this chapter). We have previously shown in a cohort of patients with chronic HF that there is a U-shaped relationship between time-weighted HbA1c and mortality – such that all-cause mortality starts to rise once HbA1c falls below 8% (Figure 6). Our work showed that this inverse relationship between mortality and HbA1c below 8% was most pronounced with the use of insulin and sulfonylureas (SU).(83) On the other hand, agents such as metformin have been shown to improve HF outcomes such as HF hospitalisation and mortality(84-86). Newer generation therapy such as glucagon like peptide (GLP)-1 agonists have also shown potential benefits by way of lower CV mortality.(87)

There is a sense of inertia (and perhaps a degree of ignorance) amongst many physicians treating T2DM and HF which is rooted in the long-standing, but unsubstantiated, belief that merely lowering blood glucose will improve

outcomes. In this section we review the complexities (and potential dangers) of treating T2DM in patients who also have HF.

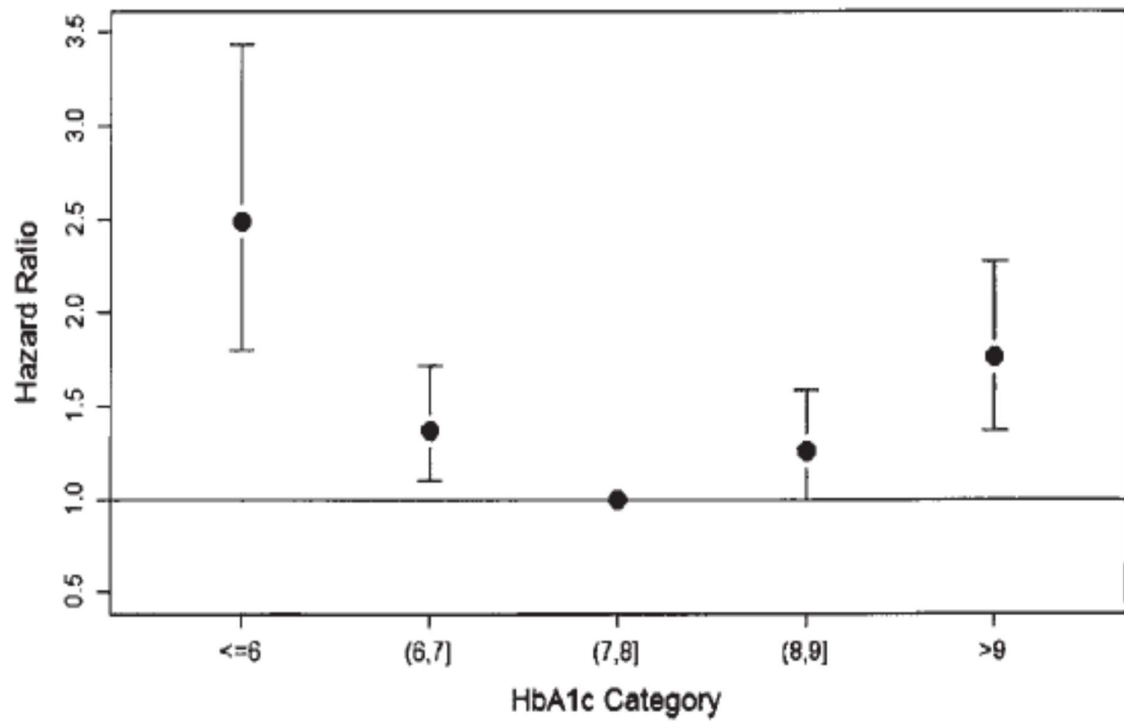


Figure 6. Risk of all-cause mortality against time-weighted HbA1c in patients with T2DM and HF.(83)

1.4.2 Sulfonylureas

SUs are insulin secretagogues and have long been used as second-line therapy in T2DM. Combined with metformin, SUs can lower HbA1c by as much as 24% compared to monotherapy but its cardiovascular safety, particularly in T2DM and HF patients remains questionable.(88) Various studies have shown an increased risk of CV events(89,90), weight gain and hypoglycaemia.(91) In a large observational study involving 91,000 patients, Tzoulaki highlighted an 18 – 30% increased risk of developing incident HF when using SU as monotherapy compared with metformin alone.(92)

Some of the suggested mechanisms of increased CV risk related to SU therapy include that of an inappropriate interaction with myocardial ATP-sensitive potassium channels disrupting cardiomyocyte ability to recover from ischaemic injury.(93) Another hypothesis relates to frequent episodes of hypoglycaemia and sympathetic activation which is associated with excess risk of arrhythmic death, particularly in the setting of HF.(94,95)

1.4.3 Thiazolidinediones

Thiazolidinediones (TZD) target the PPAR- γ receptor to increase peripheral insulin sensitivity. They were welcomed as an alternative to SUs particularly because of a lower risk of hypoglycaemia. However, a timely meta-analysis by Nissen and Wolski involving 43 trials of rosiglitazone raised concerns about excess risk of myocardial infarction by 43% and CV death by 64%.(96) This triggered further scrutiny which also found 70% higher risk of incident HF with the use of TZDs.(97) These and work by others led the US-FDA to issue a black box warning regarding the use of rosiglitazone in patients with risk of IHD and the contraindication of prescribing any TZD in the setting of NYHA functional class III-IV HF.(98)

The mechanisms of increased coronary artery disease remain controversial but revolves around its potential effect on cholesterol metabolism and the increase in serum LDL cholesterol levels.(99) The HF risk is more established and has been attributed to an increase in sodium and water retention.(100)

1.4.4 DPP-IV-Inhibitors

DPP-IV inhibitors are classified as incretin modulators, which prolong the duration of action of GLP-1 by preventing its breakdown. These drugs modestly reduce HbA1c by an average of 0.7% (101), prolong beta-cell survival, are not associated with hypoglycaemia and are weight neutral.(102) After the CV concerns raised by TZDs, the US-FDA mandated CV outcome trials for all anti-diabetic therapy which prompted further investigation into this drug class. This came in the form of three large CV outcomes trials; the Sitagliptin Cardiovascular Outcomes Study (TECOS) (103), Does Saxagliptin Reduce the Risk of Cardiovascular Events When Used Alone or Added to Other Diabetes Medications (SAVOR - TIMI 53) (104) and Cardiovascular Outcomes Study of Alogliptin in Patients With Type 2 Diabetes and Acute Coronary Syndrome (EXAMINE) (105), which set out to test the CV efficacy of sitagliptin, saxagliptin and alogliptin respectively.

All three trials were neutral in their primary outcome of major adverse cardiovascular events (MACE). However, in the case of SAVOUR-TIMI 53, saxagliptin was associated with a 27% increased risk of HF hospitalisation and 22% excess CV mortality risk. A further meta-analysis of more than 95,000 patients of 14 trials studying various glucose-lowering agents revealed those on DPP-IV-inhibitors had a 25% increased risk of developing incident HF (HR 1.25, 95% CI 1.08 -1.45).(106)

1.4.5 Insulin

Insulin plays a central role in diabetes; T2DM management involves either increasing the body's sensitivity to it, stimulating increased secretion from the pancreas or exogenous replacement of insulin. Exogenous administration of insulin has the most potent and direct effect on lowering blood glucose levels. However, as discussed above, this does not necessarily imply improved macrovascular benefits.

The increased risk of hypoglycaemia events, sodium retention and weight gain has raised concerns around its use in patients with HF, however there has been no clinical trial to date specifically designed to investigate this. In a wide-ranging analysis of four large HF clinical trials involving 24,000 patients and a further 100,000 patients with HF from an administrative database, Cosmi and colleagues found that the use of insulin (alone or in combination with other agents) resulted in increased risk of all cause death HF hospitalisation by 27% and 23% in the trial population, while the patients from the administrative database saw increased risk by 102% and 42% for the same.(107)

These signals of increased morbidity and mortality with the use of 'mainstay' anti-diabetic agents in the context of HF is concerning. It underscores the importance on focusing, not merely on glycaemic efficacy, but instead on a drug's efficacy in reducing CV risk. Indeed, current generation anti-diabetic agents are addressing this head-on, most exciting of all is the newest member of the group; the SGLT2-inhibitor class.

1.5 SODIUM-GLUCOSE CO-TRANSPORTER 2 INHIBITORS

1.5.1 Glucose Transporters

There are two broad classes of glucose transporters in man; the facilitated transporters/uniporters known simply as glucose transporter (GLUT) and the active transporters or symporters called the sodium-glucose linked cotransporter (SGLT) family. There are 14 known GLUT proteins classified into three subclasses according to similarities in their genetic sequences.(108) They all function in an energy independent manner, allowing bidirectional transport of their substrate across the cellular membrane. They each exhibit different transport kinetics that may be symmetric or asymmetric, which is outside the scope of this work.(109)

SGLT proteins, on the other hand, mediate thermodynamically-coupled transport of sodium and glucose across the plasma membrane in an ATP-dependent mechanism. The first description of this active transport of glucose was proposed by Robert Crane at the Symposium on Membrane Transport and Metabolism in Prague in 1960. (Figure 7) In his model, which has been validated and is still in use today, the 'active' transport of glucose is facilitated by the movement of sodium down its concentration gradient via the SGLT protein. The sodium gradient is maintained by a Na^+/K^+ pump, which is where the energy expenditure occurs - the movement across the SGLT protein itself doesn't require energy.(110)

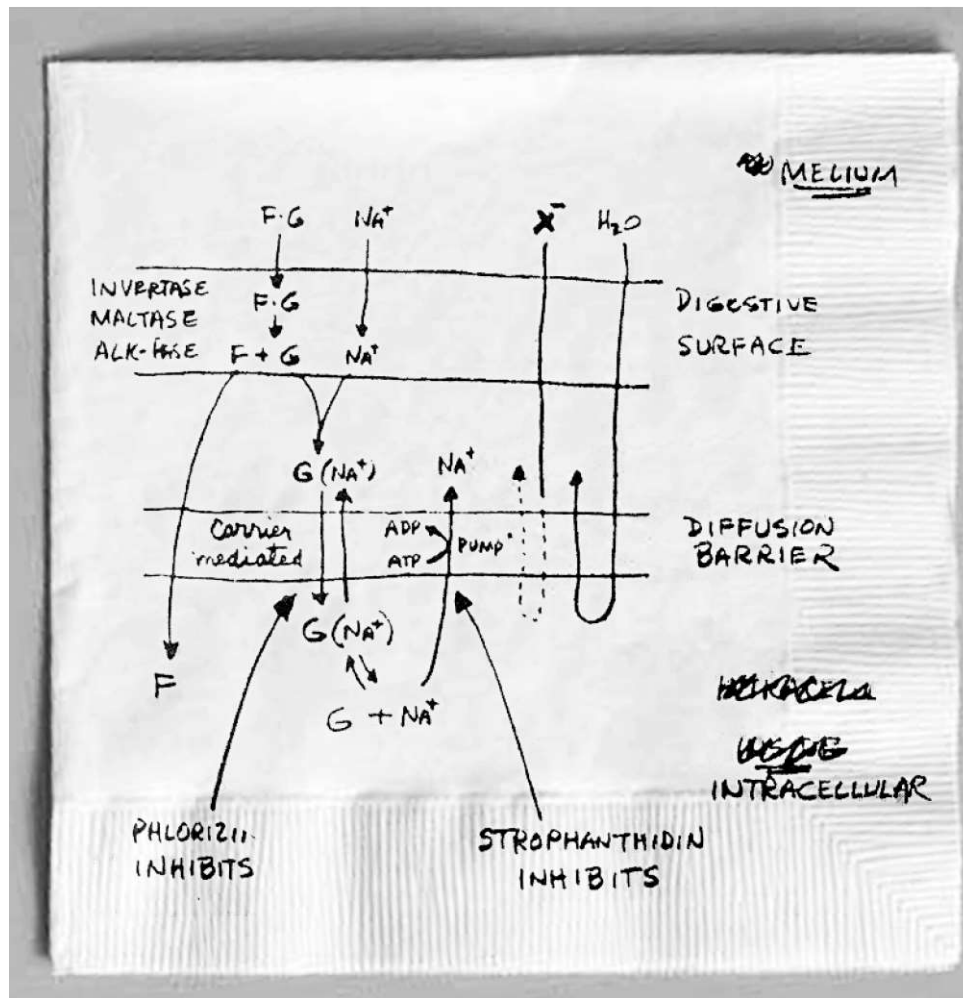


Figure 7. Drawing by Robert Crane of his cotransport model on 24 August 1960 in Prague(111)

A mechanical model for the sodium and glucose coupled transport has been described as a six-state rapid equilibrium, alternating access model. (Figure 8) States 1 to 3 are outward facing, while 4 to 6 face inward. In the unloaded state the carrier is outward facing, has a negative charge and low affinity to glucose. Extracellular sodium binding to the carrier results in a conformational change that allows glucose binding. The tertiary structure of sodium, glucose and carrier then undergoes a conformational change that faces inwards. In this

state, the ligands are released intracellularly. With the release of the positively charged sodium ion, the carrier returns to a negative charge which results in a conformational change back to an outward facing state.

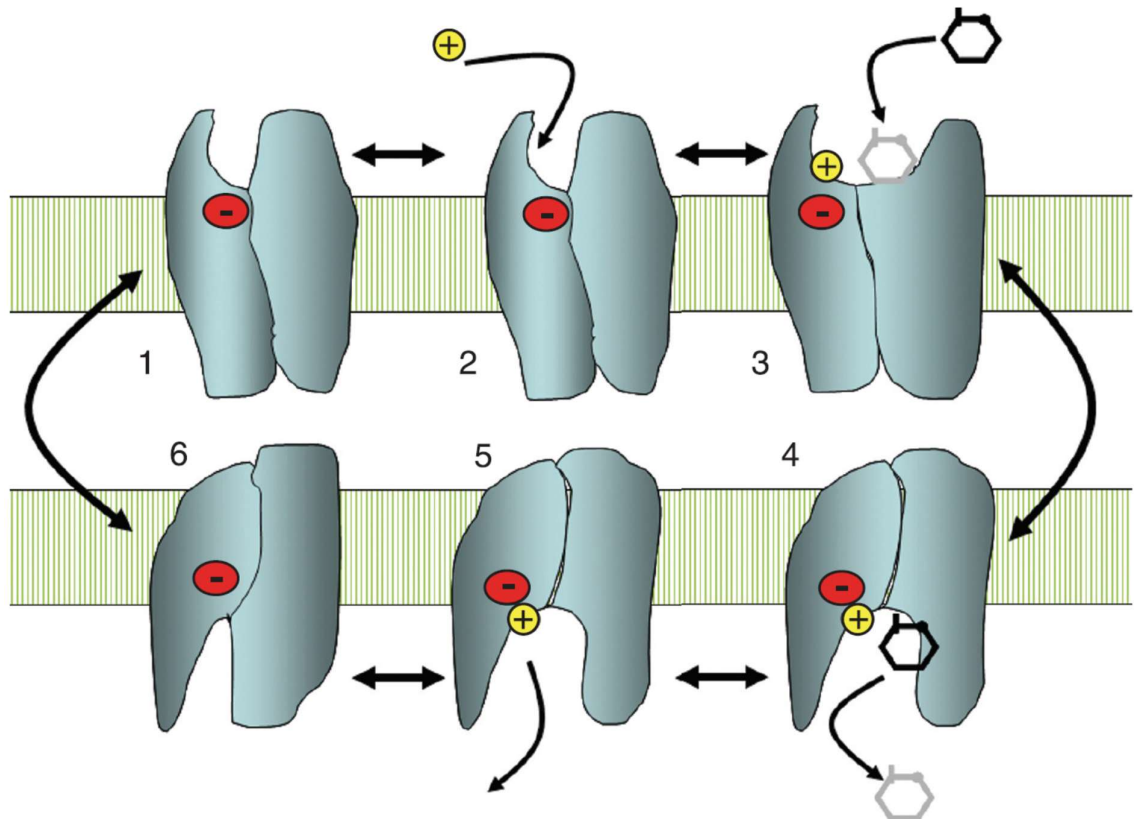


Figure 8. Mechanical model of sodium-glucose cotransport.(112)

There are six known isoforms of the SGLT protein, identified numerically from SGLT1 to SGLT6 and they can be found in varying concentrations throughout the human body in organs such as the kidneys, gut, liver, muscles, brain and heart. SGLT1 is predominantly found in the intestinal brush border but can also be found in the distal (S3) segment of the proximal convoluted tubules (PCT). SGLT3 has the most diffuse distribution throughout the body and is not strictly responsible for glucose transport per se but acts as a glucose sensor instead. The isoform of interest in this work is the SGLT2, which is concentrated in the first two segments (S1 and S2) of the PCT located in the renal cortex. mRNA expression assays have shown that SGLT2 proteins are almost exclusively expressed in the kidneys, although they may also be found in the pancreatic alpha cells, testes, prostate, heart and cerebellum. (Figure 9) (113,114)

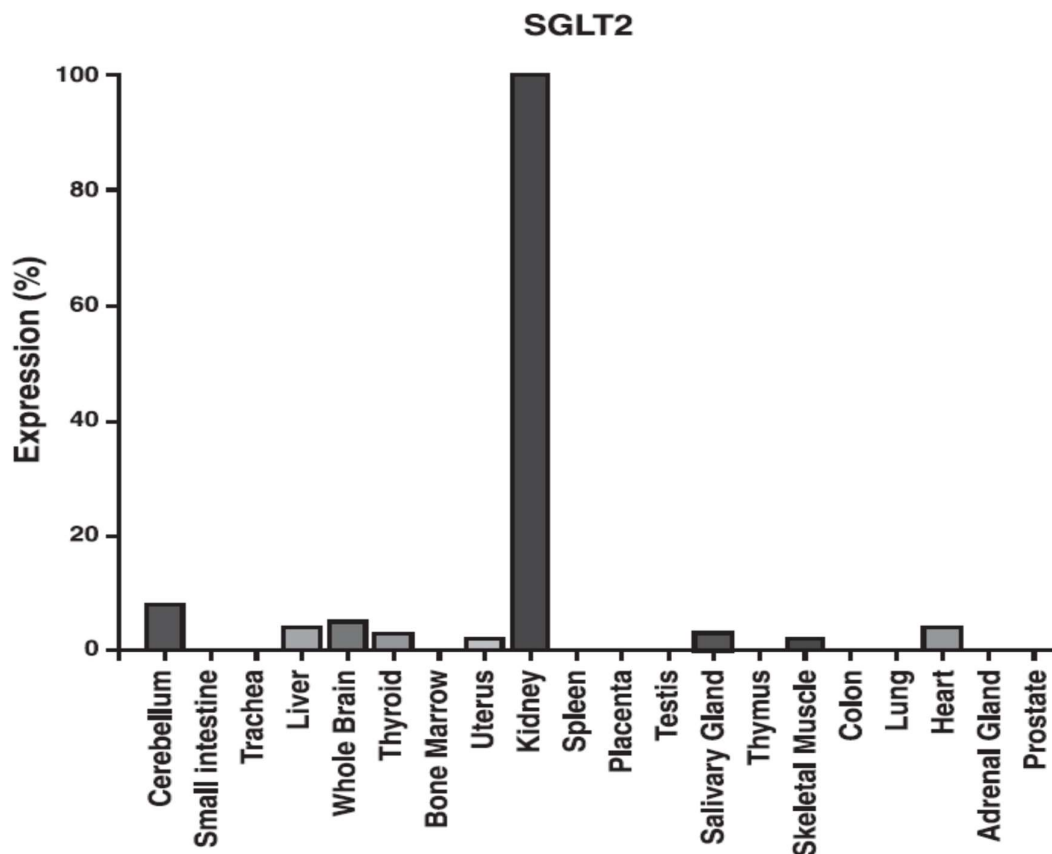


Figure 9. Expression of SGLT2 mRNA in human tissue samples.(112)

1.5.2 Renal glucose handling physiology

The glomeruli of a healthy individual filter approximately 180g of glucose a day (approximately 30% of daily energy intake) and virtually all is reabsorbed with <1% being excreted.(113) The transport proteins responsible for this are the SGLT1 and SGLT2 transporters. SGLT2 is a low affinity, high capacity transporter that is responsible for approximately 90% of glucose reabsorption in the proximal segment of the PCT, while the remainder is performed by the high affinity, low capacity SGLT1 in the distal segment of the PCT. Renal tubular glucose reabsorption is a multistep process starting with SGLT-mediated transport of glucose from the tubule into the tubular epithelial cells. This is followed by facilitated diffusion, down the concentration gradient, across the basolateral membrane into the peritubular capillaries via GLUT.

(Figure

10)

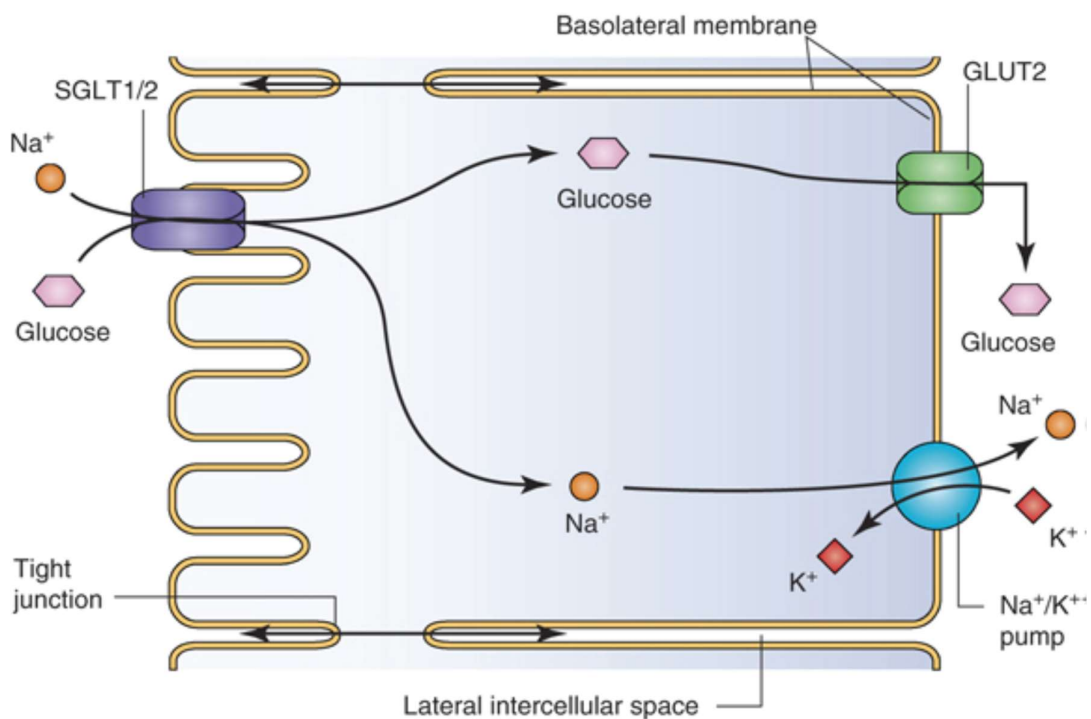


Figure 10. Physiology of renal tubular glucose reabsorption.(115)

A healthy adult is able to handle renal tubular glucose reabsorption demands up to a blood glucose level of around 12 mmol/L.(116) Although there is considerable variation in this threshold, once an individual develops T2DM, it is routinely breached, resulting in glycosuria. In such conditions, the kidney responds by upregulating SGLTs and GLUTs; Rahmoune and colleagues performed an elegant study isolating and culturing human exfoliated proximal tubular epithelial cells from fresh urine. They demonstrated for the first time that isolates from patients with T2DM expressed significantly more SGLT2 and GLUT2 proteins than healthy individuals. They also showed increased glucose uptake in the cultured isolates of these cells from the T2DM patients.(117) This increase in the renal glucose reabsorption threshold is an evolutionary response in an attempt to conserve as much of this energy substrate, but is clearly counter-productive in T2DM as it sustains the hyperglycaemic state. Hence why there has been such interest in trying to lower this threshold by blocking SGLTs.

1.5.3 Pharmacology of SGLT2-inhibitors

In 1835 French chemists first isolated the flavonoid phlorizin from the bark of an apple tree. Later observations by von Mering showed that doses greater than 1 gm produced glycosuria. It achieves this by competing with D-glucose to non-selectively bind to both SGLT1 and SGLT2 proteins. Due to its effects on the SGLT1 protein in the intestine, phlorizin also causes osmotic diarrhoea, making it a poor pharmaceutical candidate.(118)

It was only in 1999 that a team of Japanese researchers were able to modify the chemical structure of phlorizin to increase its selectivity towards SGLT2 while allowing for oral administration.(119) Since then there has been further evolution and distilling of this modification culminating in the current generation of highly selective SGLT2-inhibitors. As SGLT2 transporter proteins are predominantly renally expressed, these inhibitors selectively act on the kidneys to cause glucosuria and natriuresis as a result of competitive blockade of the SGLT2 protein. (Figure 11) Canagliflozin was the first SGLT2-inhibitor to be approved for use in T2DM by the FDA in 2013, followed by dapagliflozin and empagliflozin in early and late 2014 respectively.(120-122)

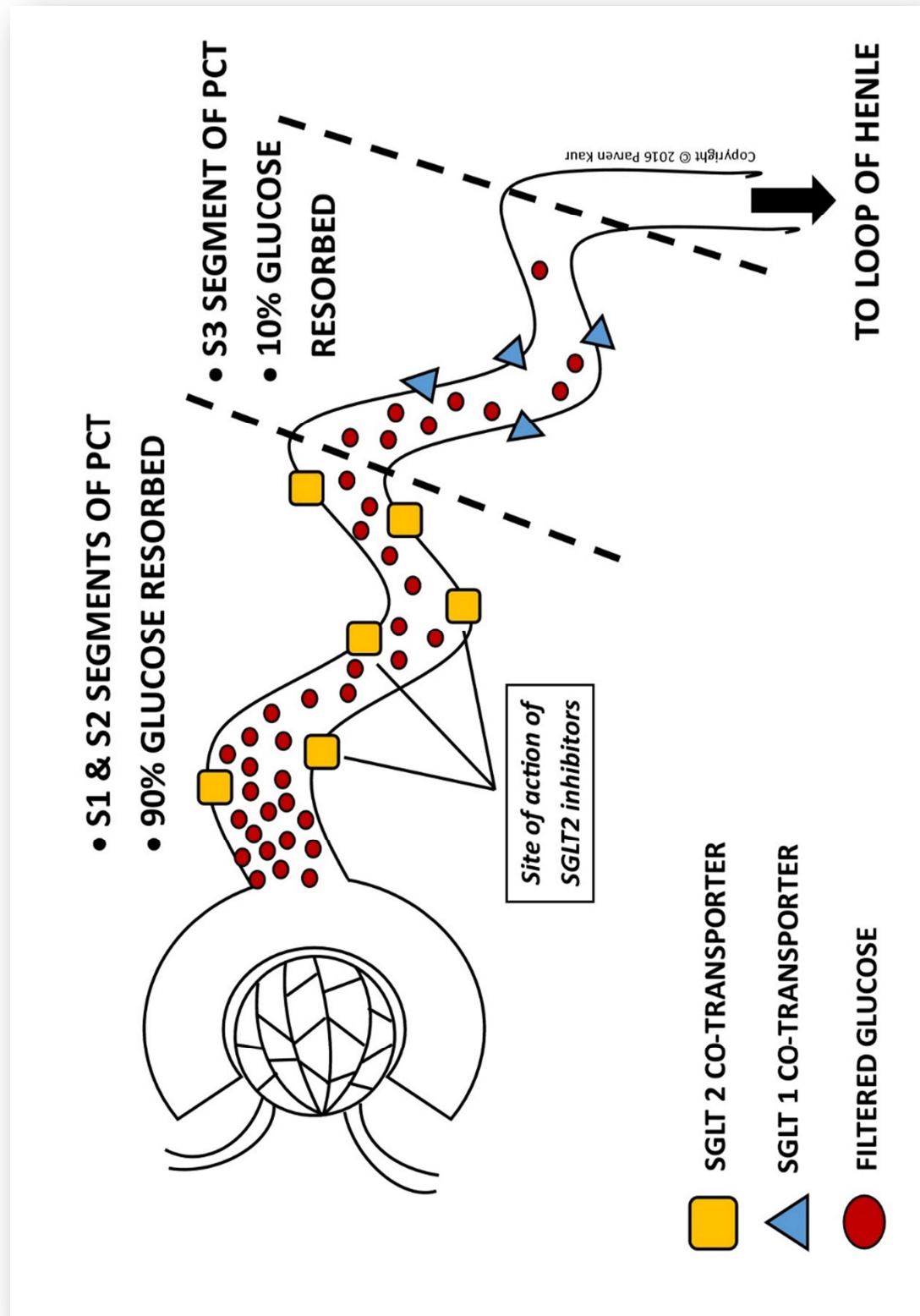


Figure 11. Mechanism of action of SGLT2-inhibitors on nephron(1)
 Reproduced with thanks to Parven Kaur

SGLT2-inhibitors result in a glycosuric effect of approximately 60-90 g/day in individuals with normal renal function.(114) Each agent has its own kinetics and has unique selectivity to different SGLT isoforms. (Figure 12) For instance, empagliflozin is most selective toward SGLT2s, while canagliflozin is the least selective, and has a modest SGLT1-inhibiting effect in the distal PCT and intestines.(123) This raises interesting questions, for instance could canagliflozin be used in more obese patients with poorer glycaemic control as it is able to block intestinal uptake of glucose and augment the glycosuric effect by blocking distal PCT reabsorption of glucose (which is enhanced due to increased glucose delivery from the inhibition of the more proximal SGLT2s)? These questions will still need to be studied, particularly the potential off-target effects of blocking SGLT1 which is more widely distributed than SGLT2.

10mg of Dapagliflozin, the drug used in the REFORM trial, has an oral bioavailability of 78% and its pharmacokinetics is unaffected by age, gender, ethnicity, weight and food consumption.(114) Other pharmacokinetic features have been summarised in Figure 12. As dapagliflozin is predominantly excreted by the kidneys, worsening renal function increased drug concentrations, in severe cases, by up to 80%. However, there was reducing efficacy with regard to its glucose-lowering effects with declining renal function.(114) Mild to moderate liver impairment does not affect dapagliflozin's pharmacokinetics but in cases of severe impairment, halving the dose is recommended.(124)

	Dose (mg)	IC ₅₀ SGLT1 vs 2	T _{max} (h)	C _{max}	t _{1/2} (h)	Population and administration regimen
Selective SGLT2 inhibitors						
Dapagliflozin	10	1390 vs 11 nmol/L Ratio ~1200:1	15–20	~160 ng/mL	~13	Healthy individuals steady state
Canagliflozin	100	910 vs 2.2 nmol/L Ratio 160:1	~1.5	~1.0 ug/mL	10.6	Type 2 diabetes single dose
	300	910 vs 2.2 nmol/L Ratio 160:1	~1.9	2.7 ug/mL	13.1	Type 2 diabetes single dose
Empagliflozin	10	8300 vs 3.1 nmol/L	1.5	259 nmol/L	13.2	Type 2 diabetes steady state
	25	8300 vs 3.1 nmol/L	1.5	687 nmol/L	13.3	Type 2 diabetes steady state
Ipragliflozin	150	254 fold selectivity for SGLT2 vs SGLT1	~1	2493 ng/mL	17.8	Healthy individuals

Figure 12. Pharmacokinetics of various SGLT2-inhibitors.(114)

An important pharmacodynamic feature of SGLT2-inhibitors is the very low hypoglycaemia risk. This is due to two inherent ‘safety features’ of SGLT2-inhibition. Firstly, by selectively blocking SGLT2 transporters that happen to be located in the proximal part of the PCT, there is increased glucose delivery downstream, resulting in increased glucose uptake by SGLT1 transporters located in the distal part of the PCT. Work on genetic knockout mice and human embryonic kidney cells show this increased glucose load to the distal PCT unmask the transport capacity of SGLT1 and fully accounts for the residual glucose uptake in SGLT2-inhibition.(125,126) This essentially means that the effect of SGLT2-inhibition is cancelled out by increased SGLT1 activity once the glomerular-filtered load of glucose reaches approximately <80g /day. The second failsafe mechanism comes from metabolic counter regulation. Unlike other anti-diabetic agents that act directly on the insulin/incretin pathway, this metabolic pathway is unaffected by SGLT2-inhibition. As will be discussed in chapter 1.6.5, SGLT2-inhibitors result in reduced insulin secretion

and increased plasma glucagon levels (resulting in hepatic gluconeogenesis) as well as shifting substrate utilisation away from carbohydrates toward lipid metabolism. These shifts in metabolism inherently prevent blood glucose levels from falling too low. These characteristics in the pharmacodynamics of this drug class has meant that hypoglycaemia has only been reported when SGLT2-inhibitors were used in combination with other anti-diabetic drugs, but not as monotherapy.(127)

Another unique feature of SGLT2-inhibitors is their insulin-independent activity. Unlike other anti-diabetic agents that act either by increasing sensitivity to insulin or by manipulating the incretin pathway to increase insulin secretion, SGLT2-inhibitors act by a completely distinct mechanism that is unaffected by insulin levels or sensitivity. This allows the efficacy of SGLT2-inhibitors to remain unchanged with progressive beta-cell dysfunction or worsening insulin resistance which is typical of T2DM. This also means that SGLT2-inhibitors can be used as synergetic agents to other glucose lowering drugs as they act on a different pathway.(116,128) There are authors that argue this insulin-independent activity combined with the insulin-lowering effects (through sustained glycosuria) of SGLT2-inhibitors allow them to preserve pancreatic beta-cell function, thereby delaying the progression of T2DM, although this will need further confirmation.(129)

1.6 CARDIO-METABOLIC EFFECTS OF SGLT2-INHIBITION

1.6.1 Improved glycaemic control

SGLT2-inhibitors have been shown to reduce HbA1c levels between 0.4% to 1.1% depending on baseline HbA1c and type of SGLT2-inhibitor used. Canagliflozin which also has modest SGLT1-inhibitory activity, seems to have a slight advantage over the other agents.(130-132) 10 mg once daily of dapagliflozin reduces HbA1c between 0.58% to 1.11% in patients with T2DM.(133)

Compared to other anti-diabetic agents, SGLT2-inhibitors seem to either be as effective or marginally superior in reducing HbA1c but are associated with lower risk of hypoglycaemia. When empagliflozin was compared to the sulfonylurea glimepiride as add on therapy to metformin, there was a more prominent HbA1c reduction with glimepiride initially, but at the end of the 2-year observation period patients in the empagliflozin group saw an adjusted mean difference in HbA1c of -0.11% (95% CI -0.19 to -0.02; p=0.0153 for superiority) compared to the glimepiride group. Incidence of confirmed hypoglycaemia was 2% in the empagliflozin group vs 24% in the glimepiride group over the same duration.(134) In a meta-analysis of 25 trials involving nearly 15,000 patients Wang et al. showed that SGLT2-inhibitors were superior to DPPIV-inhibitors as monotherapy or add-on to metformin therapy in patients with T2DM. They found a weighted mean difference in HbA1c of 0.13% (95% credible interval, 0.04%-0.22%, P = 0.005) in favour of SGLT2-inhibitors, and no significant difference in hypoglycaemic events.(135) A real-world observation of 411 patients receiving add on SGLT2-inhibitor to insulin

due to poor glycaemic control found patients on >200 IU/day insulin had a 23 IU/day reduction with canagliflozin and 71 IU/day reduction with dapagliflozin after 6 months.(136)

1.6.2 Weight reduction

A meta-analysis of 39 trials involving 25,000 individuals showed canagliflozin 300mg, empagliflozin 25mg and dapagliflozin 10mg all at a once daily dosing resulted in a weight loss of 2.66kg, 1.80kg and 1.81kg respectively compared to placebo.(137)

Early hypotheses suggested that the weight loss observed in the use of SGLT2-inhibitors were solely due to the loss of calories from glycosuria. However, there was a consistent underestimation of weight loss from the measured amount of caloric loss. In an edifying study, Ferrannini and colleagues demonstrated that the glycosuria induced an adaptive increase in calorie intake, accounting for the deficit between the expected and observed weight loss.(138)

Certainly, in the initial stages of SGLT2-inhibitor therapy, caloric loss from glycosuria and fluid loss from osmotic diuresis are the two primary contributors to weight loss. However, these effects are transient and are usually lost beyond first 12 weeks, likely due to compensatory mechanisms.(139)

More sustained weight loss in SGLT2-inhibitor therapy is due to fat loss. As will be discussed in the subsequent section, SGLT2-inhibitors induce lipolysis due to alterations in the insulin-glucagon ratio. Studies utilising dual-energy x-ray absorptiometry (DEXA) have shown approximately 70% of the sustained weight loss in individuals with T2DM treated with dapagliflozin over 2 years was attributable to loss of body fat. An MRI sub-study revealed that there were numerically greater reductions in visceral adipose tissue compared to

subcutaneous adipose tissue in the dapagliflozin group.(140) This is potentially important because visceral adipose tissue has been implicated in a number of diseases including IHD, hypertension, T2DM and insulin resistance, colon, breast and prostate cancer and increased in-hospital mortality.(141)

1.6.3 Natriuresis and diuresis

Inhibition of SGLT2 transporters in the proximal convoluted tubules results in glycosuria and natriuresis which, in turn, causes osmotic diuresis. This natriuretic and diuretic effect of SGLT2-inhibition has been clearly documented.(142) An increase of mean urine volume of between 100-500 mls/day has been reported in various phase 3 trials.(143)

This effect is the putative mechanism of CV benefit by way of volume reduction resulting in lower BP, reduced pre- and afterload on the heart and decongestion in the case of HF.(144) Interestingly, SGLT2-inhibitors may have unique abilities over and above classical agents such as thiazide and loop diuretics. In a small study comparing dapagliflozin and hydrochlorothiazide, a 7% volume contraction and 2.2 percentage-points increase in haematocrit was seen with dapagliflozin over a 12-week period. However, the hydrochlorothiazide group had significantly lower 24-hrs mean BP which was not seen in the dapagliflozin group.(145) In a different mathematical modelling study, the authors found dapagliflozin to be far more efficient at removing interstitial fluid volume without compromising intravascular volume. The authors calculated that SGLT2-inhibitors can achieve this effect by promoting greater electrolyte-free water clearance compared to bumetanide, and in doing so, achieve a 200% reduction in interstitial volume compared to blood volume while bumetanide was only able to achieve 78% reduction in interstitial fluid compared to blood volume. This differential effect on volume regulation could be a game-changer in patients with HF who are frequently grossly volume

overloaded in the interstitium while simultaneously being intravascularly depleted (with low blood pressure and reduced cardiac output).(146)

Finally, another unique feature of SGLT2-inhibitor-related diuresis is that there is no reflex sympathetic activation as is usually seen with the other diuretic drug classes.(147) Investigators have yet to fully clarify why this the case, but one could imagine the above discussion around the preserved intravascular volumes may be a potential explanation. Some authors have also speculated that this is an indirect effect of increased sodium delivery to the macula densa which is a specialised sodium sensor at the junction between the loop of Henle and distal convoluted tubule, forming part of the juxtaglomerular apparatus. The rise in tubular sodium concentration (as a result of SGLT2-inhibition) triggers a tubuloglomerular feedback mechanism that increases afferent arteriolar vasoconstriction to reduce renal blood flow into the glomerulus and reduces renin release (thus indirectly blunting sympathetic outflow) because of the 'perceived' high blood sodium levels as measured by the filtered sodium concentration within the tubules.(148)

This is an important feature of SGLT2-inhibition as sympathetic overactivity has been associated with higher mortality in patients with HF(149) and this could perhaps be part of the explanation why diuretics have not been shown to have a mortality benefit in HF but SGLT2-inhibitors have. (See section 1.7)

1.6.4 Blood pressure reduction

SGLT2-inhibitors also reduce systolic and diastolic BP. A meta-analysis of 43 trials that reported BP change involving 22,000 patients showed SGLT2-inhibitors reduced the weighted mean systolic BP by -2.46 mmHg (95% CI -2.86 to -2.06) and weighted mean diastolic pressure was reduced by -1.46 mmHg (95% CI -1.82 to -1.09). The majority of the trials (n=23) had a duration of between 12-24 weeks. Interestingly, empagliflozin was most potent for systolic BP reduction (-2.59 mmHg [95% CI -2.70 to -2.49]), while canagliflozin reduced diastolic BP the most (-2.23 mmHg [95% CI -2.30 to -2.16]).(150)

In another meta-analysis looking at 24-hours ambulatory BP, SGLT2-inhibitors were found to reduce systolic and diastolic BP by -3.76 mmHg (95% CI, -4.23 to -2.34) and -1.83 mmHg (95% CI, -2.35 to -1.31), respectively. Dapagliflozin performed best in 24-hour mean systolic BP reduction (-3.73 mmHg [95% CI -6.38 to -1.07]), while empagliflozin reduced 24-hour mean diastolic BP the most (-1.51 mmHg [95% CI -2.91 to -0.11]). (151)

Importantly, this blood pressure lowering effect is independent of glycaemia; it has been observed in healthy individuals without T2DM (systolic BP reduction ~ 2.7mmHg) (152) as well as in patients with moderate renal dysfunction, despite minimal reduction in HbA1c on SGLT2-inhibitor therapy.(143,152)

The proposed mechanisms of BP reduction are threefold; firstly, plasma volume contraction from osmotic diuresis and natriuresis. Secondly by improved vascular physiology by way of reduced smooth muscle tone (through voltage-gated potassium (Kv) channels and protein kinase G activation),

reduced arterial stiffness and improved endothelial function. Thirdly, as an indirect effect of weight reduction.(153,154)

1.6.5 Lipolysis and increased ketone body production

The proximal convoluted tubules of the renal nephron contain the highest concentration of SGLT2 transporters in the body, however SGLT2 transporters can also be found in numerous other organs such as the pancreatic alpha cells (but not in the beta cells)(155), testes, prostate, heart and brain.(112)

In the pancreas the SGLT2 transporter is responsible for glucose uptake into the alpha cells which produces glucagon. When the SGLT2 transporters of the alpha cells are inhibited, it mimics a hypoglycaemic state and induces glucagon secretion via K_{ATP} channel activation and membrane repolarisation.(155) Simultaneously, as discussed in section 1.6.1 above, the glycosuric effect of SGLT2-inhibition in the kidney lowers blood glucose levels thereby reducing insulin production / requirements. This combination of lower insulin and higher glucagon levels shifts metabolism towards increased lipolysis and mild ketogenesis.(156) This is the same triggering mechanism for diabetic ketoacidosis; the difference being in that case the reduction in the insulin : glucagon ratio is very large and rapid (occurring over hours to days) compared to SGLT2-inhibition where it is of a smaller magnitude and more protracted (occurring over weeks), resulting in a gradual and modest rise in ketone bodies.

This effect of increased lipolysis and a modest rise in ketone bodies have beneficial effects by way of weight loss and improved myocardial energetics. This will be discussed in greater detail in chapters 4.3.1 and 4.4.

1.6.6 Increased haemoglobin and haematocrit

SGLT2-inhibitors increase haemoglobin (Hb) and haematocrit (Hct).(157) The early explanation for this was that of haemoconcentration from diuresis, however this was disproved when a discordance between Hct and serum osmolality was observed.(158) Further work into this revealed on top of some haemoconcentration from diuresis, there is an increase in erythropoietin (EPO) production in patients on SGLT2-inhibitors. A 12-week study comparing dapagliflozin with hydrochlorothiazide and placebo showed increased red cell mass and EPO production,(145) while another using empagliflozin for 4 weeks showed a 60% increase in EPO production.(159)

The mechanism behind this effect was explained by Sano and colleagues; The increased glucose load in renal tubules as a result of T2DM causes an upregulation of SGLT2 transporter activity in the renal cortex. This increases the cortical oxygen demand (as tubular glucose reabsorption is an active process), generating relative cortical ischaemia. EPO-producing fibroblasts, usually found in the renal cortex, respond to this ischaemia by transforming into (non-EPO producing) myofibroblast that promote interstitial fibrosis and predispose to renal dysfunction. When an SGLT2-inhibitor reduces renal cortical energy requirements by effectively shutting down these transporters, the myofibroblasts revert back to their original state resulting in increased EPO levels.(157,160)

This increase in Hb / Hct is significant because it increases myocardial oxygen delivery and potentially plays a role in improving overall myocardial energetics (see sections 1.3.2 and 4.4). Indeed, a mediation analysis of the EMPA-REG

OUTCOME trial showed that 52% of the beneficial effect of empagliflozin on CV mortality can be attributed to its effect on haematocrit alone.(161)

Nevertheless, this effect may also be a double-edged sword as there have been concerns around increased amputations (especially with the use of canagliflozin) and the lack of stroke protection, in spite of its glowing performance in CV risk reduction. Although a causal link has yet to be established, one wonders if the increase in Hb / Hct may be a contributor?

1.7 CARDIOVASCULAR OUTCOME TRIALS

1.7.1 Overview

At time of writing, there have been three large CV outcome trials studying each of the most widely available agents in the SGLT2-inhibitor class. By order of publication, they are the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME), Canagliflozin Cardiovascular Assessment Study (CANVAS) and the Multicentre Trial to Evaluate the Effect of Dapagliflozin on the Incidence of Cardiovascular Events (DECLARE-TIMI 58). There has also been a meta-analysis of all three trials providing a comprehensive overview of the CV protective effects of this drug class.

In this chapter we briefly discuss the key features of each of these trials to form an understanding of the potential effects of this drug class on CV outcomes. Even though the REFORM trial was conceived before any of these trials published their findings, many of the results from our work supplements and builds on their findings and forms the basis of ongoing and future work in this field.

1.7.2 EMPA-REG OUTCOME

The EMPA-REG OUTCOME trial (162) published in 2015 studied 7000 individuals with T2DM over a mean follow up period of 3 years. Patients were randomised to either empagliflozin 10mg OD, 25mg OD or placebo in equal proportions.

This was the only trial, of the three, that mandated established CV disease (defined as previous MI / stroke, confirmed coronary or peripheral arterial disease of >50% occlusion) along with T2DM as the inclusion criteria. This meant that the EMPA-REG cohort had the highest risk profile of the three SGLT2-inhibitor CV outcome trials.

The average age was 63 years, majority white male population with nearly 60% having T2DM for at least 10 years. Approximately $\frac{3}{4}$ of patients had coronary artery disease, and approximately 20% each with stroke and peripheral arterial disease. Only 10% had a prior diagnosis of HF, but it is important to note that this classification was simply made by asking trial patients if they had a diagnosis of HF and no formal confirmation (e.g. by measuring natriuretic peptides or performing an echocardiogram) was required. Majority of patients were on ACE-i/ARB (81%) and beta-blockers (65%). Interestingly 44% were on diuretic therapy suggesting that, perhaps, there were more patients with HF in the trial.

Pooled analysis (both arms of empagliflozin together vs placebo) showed relative risk reduction in MACE (CV death, nonfatal MI / stroke) by 14%; HR 0.86; (95% CI 0.74-0.99); p=0.04. Rather unexpectedly there were also striking

reductions in all-cause mortality (HR 0.68; 95% CI 0.57-0.82; $P<0.001$), CV mortality (HR 0.62; 95% CI 0.49-0.77; $P<0.001$) and HF hospitalisation (HR 0.65; 95% CI 0.50-0.85; $P=0.002$) There was an overall 5/2 mmHg blood pressure reduction and an approximately 2kg weight loss in the empagliflozin arm.(163)

1.7.3 CANVAS

The CANVAS trial (164) was published in 2017. It recruited around 10,000 individuals with T2DM and followed them for an average of nearly 4 years. Patients were randomised to either canagliflozin 100mg OD, 300mg OD or placebo in a 1:1:1 fashion.

CANVAS allowed both established and at-risk CV disease individuals to be recruited. An individual was considered at risk for CV disease if they had 2 or more of the following: long-standing T2DM (≥ 10 years), poorly controlled BP while on at least 1 BP-lowering agent, cigarette smoker, documented micro/macro -albuminuria or documented HDL < 1 mmol/l.

The average age was 63 years, majority white male population with an average duration of T2DM of 13.5 years. Approximately 60% of the cohort had established CV disease, while the remainder were at-risk of CV disease. Once again, only a small proportion (14%) had a prior diagnosis of HF. In spite of the lower risk profile, the baseline medications of ACE/ARB (80%), beta-blockers (54%) and diuretics (44%) were similar to EMPA-REG OUTCOME.

Pooled analysis showed lower MACE (HR 0.86; 95% CI, 0.75 to 0.97; $p=0.02$) and lower HF hospitalisation (HR 0.67; 95% CI, 0.52 to 0.87) which are comparable to the findings of EMPA-REG OUTCOME. There was no significant reduction in mortality.

The mean difference in body weight was -1.60 kg (95% CI, -1.70 to -1.51), while difference in systolic blood pressure was -3.93 mm Hg (95% CI, -4.30

to -3.56), and diastolic blood pressure was -1.39 mm Hg (95% CI, -1.61 to -1.17). There was less progression to albuminuria and amongst those already with albuminuria, they were more likely to regress; 0.73 (95% CI, 0.67 to 0.79) and 1.70 ; (95% CI, 1.51 to 1.91) respectively. Importantly, there was also 40% less need for renal-replacement therapy, or death from renal causes in the canagliflozin group; 0.60 ; (95% CI, 0.47 to 0.77).

There were some potential safety concerns raised in the CANVAS trial, other than the commonly accepted genital infection and euglycaemic ketoacidosis. There was a higher risk of amputation; 1.97 ; (95% CI, 1.41 to 2.75) and patients with the highest risk for amputation were those with a previous history of amputations or documented peripheral vascular disease. More fractures were also noted in the canagliflozin group; 1.26 ; (95% CI, 1.04 to 1.52). It is still unclear if this is a class effect or a problem specific to canagliflozin as it was not seen in trials for other members of the class.

1.7.4 DECLARE-TIMI 58

The most recent study to be published is the DECLARE - TIMI 58 trial.(165) It randomised 17,000 patients with T2DM to dapagliflozin 10mg OD vs placebo and followed them for a median of just over 4 years.

The average age was 64 years, majority white men with a median duration of T2DM of 10.5 years. In contrast to CANVAS, 60% of the cohort were at risk of developing CV disease while the remaining had established disease, the majority of which (33%) was that of coronary artery disease. Only 10% had a diagnosis of HF. Once again, baseline medications were comparable to the other trials with 81% on an ACE-I / ARB, 52% on beta-blockers and 41% on diuretics even though this was a much lower risk group.

Dapagliflozin had no effect on MACE (HR 0.93; 95% CI 0.84 - 1.03; $p = 0.17$) or CV death (HR 0.98; 95% CI 0.82 - 1.17). However, there were significantly lower hospitalisations for HF (HR 0.73; 95% CI 0.61 - 0.88). In this study as well, investigators noted a reduction in mean weight by 1.8 kg (95% CI 1.7 - 2.0), systolic BP by 2.7 mmHg (95% CI, 2.4 - 3.0), and diastolic BP by 0.7 mmHg (95% CI, 0.6 to 0.9).

A recently published subgroup analysis of the DECLARE TIMI-58 trial(166) showed that dapagliflozin reduced CV death and all-cause mortality in patients with HFrEF (defined as a previously a documented LVEF of <45%) - HR 0.55, 95% CI 0.34 - 0.90; $p_{\text{interaction}}=0.012$ and HR 0.59, 95% CI 0.40 - 0.88: $p_{\text{interaction}}=0.016$ respectively. This was the first time the effects of an SGLT2-inhibitor were stratified by severity of LV dysfunction (as defined by LVEF).

There is a clear pattern emerging from these three trials; as the risk profile of their cohorts increase, so does the 'efficacy' of SGLT2-inhibition in reducing mortality (CV death) and morbidity (MACE). However, the reduction in HF hospitalisation remains robust and very consistent across the spectrum. Of course, this could also be a differential effect between the various agents in the class, but this is difficult to ascertain from current data. Another consistent feature of all the trials was that of weight loss and BP reduction.

1.7.5 SGLT2-Inhibitor CV Outcomes Meta-Analysis

Shortly after the completion of DECLARE-TIMI 58, a meta-analysis reviewing all three trials was published.(167)

A total of 34,000 individuals were included in the work, in which 60% had established CV disease and 11% with a diagnosis of HF. There was an overall 11% reduction in MACE (HR 0.89; 95% CI 0.83–0.96, $p=0.0014$) but subgroup analysis showed this was restricted to those with established CV disease where a 14% reduction in MACE was seen compared to no change (0%) in those with CV risk only. There was a 23% reduction in the composite of CV death and HF hospitalisation (HR 0.77; 95% CI 0.71–0.84, $p<0.0001$), which was seen equally, with or without CV disease and with or without HF. The benefits of HF hospitalisation remained robust throughout all subgroups of CV risk / disease / HF. However, there seemed to be a greater benefit in patients with more severe renal function - eGFR < 60 ml/min/1.73m² (HR 0.60 95% CI 0.47–0.77, $p<0.0001$) and eGFR 60-90 ml/min/1.73m² (HR 0.69; 95% CI 0.57–0.83, $p<0.0001$) - compared to those with normal renal function (HR 0.88; 95% CI: 0.68–1.13, $p=0.31$). Strikingly, there was a 45% reduction in the progression of renal disease (HR 0.55; 95% CI 0.48–0.64, $p<0.0001$) which was maintained regardless of CV history. (Progression of renal disease was defined differently between trials; all trials used a composite of three measures, namely, initiation of renal replacement therapy or death from renal causes or worsening renal function. The difference was in the definition of worsening renal function; defined as doubling of serum creatinine in EMPA-REG OUTCOME, 40% reduction in eGFR (based on Modification of Diet in

Renal Disease equation) in CANVAS and 40% reduction in eGFR (based on the Chronic Kidney Disease Epidemiology Collaboration equation) in DECLARE TIMI-58)

There was no signal of increased risk of stroke and the increased risk of amputations and fractures were only seen with the use of canagliflozin (recognising that this study only involved 1 trial for each drug in class, which is far from conclusive). There was a clear increase in mycotic genital infections, but these were easily treated, and recurrence was uncommon. The risk of diabetic ketoacidosis (not specified whether this was euglycemic ketoacidosis) was doubled in the SGLT2-inhibitor group compared to placebo but the absolute incidence remained very low at <0.1%.

This meta-analysis shows that SGLT2-inhibitors have primary preventive effects with regard to renal protection and prevention of HF hospitalisation but reduction in MACE is only seen in established CV disease. Nevertheless, it is important to recognise that T2DM itself significantly increases risk of renovascular disease and HF, therefore any protection conferred by SGLT2-inhibitor therapy regardless of the 'conceptual' distinction of primary or secondary prevention cannot be underestimated. This was supported by the recent joint consensus report by the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) recommending that after initiating metformin, SGLT2-inhibitor therapy should be first choice for all patients with T2DM and HF or renal disease and joint first choice with GLP-1 receptor agonists for patients with atherosclerotic CV disease.(168)

1.8 RESEARCH HYPOTHESIS

We aimed to test if the various unique characteristics of SGLT2-inhibitor are beneficial in HF by either favourably remodelling the LV and / or improving exercise capacity in patients with T2DM and HF. If so, it would strengthen the case for early initiation of SGLT2-inhibitors in this cohort. (The recent ADA / EASD recommendations had not been published at the time of conception of this trial)

The main premise behind this hypothesis is that SGLT2-inhibitors are mild diuretics which should reduce LV preload and LV end diastolic / systolic volumes, thereby resulting in LV reverse remodelling. Whether this diuretic effect will actually occur in HF where eGFR are at the lower end of normal (or mildly impaired) plus the concomitant use of chronic loop diuretic therapy, is not yet known. Secondly, SGLT2-inhibitors reduce BP which should reduce LV afterload and further contribute to LV reverse remodelling.

A beneficial effect on LV remodelling in HF has major implications on mortality in HF patients with T2DM as it is the best surrogate for disease progression and survival in patients with HF. Indeed, in 2000 the International Forum on Cardiac Remodelling published a consensus document stating that new drug treatments in HF should be assessed by their effect on LV remodelling.(169) Serial changes in LV remodelling parameters are an excellent way to assess the effect of therapeutic interventions (drugs or devices) on clinical outcomes in patients with left ventricular systolic dysfunction (LVSD).(170) Kramer et al(171) confirmed this when they meta-analysed 30 mortality trials (involving

25 drugs or device interventions) and 88 remodelling studies, showing excellent correlations between effects on LV remodelling and mortality. The odds ratio for death in the mortality trials was correlated with drug/device effects on changes in LVEF ($r=-0.51$, $p<0.001$), LV EDV ($r = 0.44$, $p = 0.002$) and LV ESV ($r=0.48$, $p=0.002$). Thus, if SGLT2-inhibitors did result in LV reverse remodelling in HF patients with T2DM, this could provide a mechanistic explanation for the mortality benefits seen in the large SGLT2-inhibitor CV outcome trials.

SGLT2 inhibitors may also improve exercise tolerance, a universal symptom of patients with HF. Firstly, SGLT2 inhibitors have been shown to cause weight loss which will improve exercise effort. Secondly, SGLT2 inhibitors may improve exercise capacity by improving insulin sensitivity. Better insulin sensitivity has been shown to improve exercise capacity in diabetic individuals (172) and in patients with HF.(173) SGLT2 inhibitors have been shown to improve insulin sensitivity in Zucker diabetic fatty rats treated with dapagliflozin(174) and empagliflozin(175). In a randomized, double blind placebo controlled study, dapagliflozin treatment improved insulin sensitivity as measured by glucose disappearance rate during hyperinsulinaemic euglycaemic clamping in T2DM patients.(176) Thus, there is a potential that SGLT2-inhibitors, by causing weight loss and improving insulin sensitivity, may improve exercise capacity in patients with HF.

In summary, we hypothesize that the unique effects of SGLT2-inhibition may result in improved haemodynamics (by way of reduced preload, afterload and BP) and metabolic function (by way of improved insulin sensitivity and weight

loss) resulting in LV reverse remodelling and improved exercise capacity which are central facets of improving morbidity and mortality in HF. At the time this study was conceived there were no data on HF (or even CV) outcomes related to the use of SGLT2-inhibitors. However, as the large SGLT2-inhibitor CV outcome trials started reporting an unexpected but striking benefit in HF hospitalisation and mortality, we recognised that this work could provide important mechanistic insights into those findings. This was of particular importance because only a small proportion of the patients of those trials (10.0% to 14.4%) actually had a diagnosis of HF,^(162,164,165) making it difficult to fully appreciate its effects in this cohort of patients, necessitating a dedicated clinical trial.

2. METHODS

2.1 FUNDING, APPROVALS AND TRIAL REGISTRATION

Funding:

The REFORM trial was funded by the European Foundation for the Study of Diabetes (EFSD) as part of their Clinical Diabetes Research Programme supported by an unrestricted grant from Astra Zeneca / Bristol-Myers Squibb.

Ethical Approval:

The REFORM trial was approved by the East of Scotland Research Ethics Committee on 19th August 2014. Reference number 14/ES/1050.

Medicines and Healthcare products Regulatory Agency (MHRA)

Approval:

The REFORM trial was approved by the MHRA on 23rd December 2014 with the EUDRA-CT number 2014-002742-42.

Trial Registration:

The REFORM trial was registered with the U.S. National Institutes of Health via the U.S. National Library of Medicine website Clinical Trials.gov with the identification number NCT02397421. The trial was also registered with the UK Clinical Research Network with the identification number 18467.

Local approval:

The REFORM trial received final approval from the NHS Tayside Research and Development Committee on 13th February 2015 with an overall greenlight to start recruitment on 5th March 2015.

2.2 STUDY DESIGN

The REFORM trial was a single centre, double blind, placebo-controlled, parallel arms clinical trial. Patients were randomised in a 1:1 ratio to either 10mg dapagliflozin once daily or placebo (microcrystalline cellulose Ph Eur over encapsulated in a hard gelatine capsule shell). They were then observed for 1 year with between 8 to 9 visits, three of which were telephone 'visits'. Details of the visit schedule and tests performed during these visits are discussed in section 2.8.

2.3 INCLUSION /EXCLUSION CRITERIA

Patients were eligible if they were:

- Aged between 18 years to 75 years with previously diagnosed T2DM
- Diagnosed with NYHA functional class I-III HF with prior echocardiographic evidence of LVSD (Calculated LVEF of <45% or at least mild LVSD on subjective 'eyeballing' assessment)
- On furosemide 80mg daily or less, or equivalent loop diuretic
- Have stable HF symptoms for at least three months prior to consent
- On stable therapy for HF for at least three months prior to consent
- Have not been hospitalised for HF for at least three months prior to consent

Patients were excluded for the following:

- screening HbA1c <6.0%
- severe hepatic disease
- renal disease defined as CKD stage 3B or worse (i.e. eGFR or CrCl <45ml/min)
- systolic BP <95mmHg at screening visit
- unable to walk to perform cardio pulmonary exercise testing or 6MWT
- malignancy (receiving active treatment) or other life-threatening diseases
- pregnant or lactating women
- any contraindication to CMR (e.g. claustrophobia, metal implants, penetrative eye injury or exposure to metal fragments in eye requiring medical attention)
- patients who have participated in any other clinical trial of an investigational medicinal product within the previous 30 days
- patients who are unable to give informed consent

2.4 COHORT SIZE AND POWER CALCULATIONS

Improvement in LV volumes have been shown to be an important marker for the efficacy of a drug / device therapy on improving heart failure survival.(170) Grothues et al. suggest a 10 mL change in LVEDV and LVESV as clinically significant and have reported the SD for the mean difference of LVEDV and LVESV as 7.6 and 7.4 respectively.(177) Therefore, to detect a 10 mL change in LVEDV and LVESV (primary endpoint) with 90 % power and α error (p value) of 0.05, a sample size of 13 and 12 respectively were required per arm.

LV mass is also an important determinant of survival in patients with HF and a 10 g reduction in LV mass has been shown to be clinically meaningful.(170,177) The reported SD for 10 g mean change in LV mass in the HF population is 9.6 using CMR, implying 20 patients were required per arm.(177)

Kramer et al demonstrated the change in LV EF which best discriminated between drugs with positive and neutral effects on mortality was 3%. This degree of improvement in LV EF was also associated with a 20% improvement in mortality.(171) This is echoed by Grothues et al who recommend this magnitude of change be used to power studies. Our previous CMR experience has shown an in-house and published SD of the change in LV EF within individuals over time as 3.75% for both active and placebo therapies.(178) Therefore in order to have 80 % power at $p < 0.05$ to detect a $\geq 3\%$ change in LVEF in a parallel group study, 26 patients per group were needed.

As this was a discovery trial, we aimed to ensure the trial was adequately powered to detect all the clinically relevant markers of LV remodelling (LV volumes, mass and EF). Therefore, we aimed to recruit a minimum of 52 patients (26 patients per arm) to provide at least 80 % power (α error of 0.05) to detect clinically significant changes in LVEDV, LVESV, LV mass and LV EF.

2.5 INFORMED CONSENT AND RECRUITMENT

Majority of patients were recruited from the Tayside area. We accessed the Tayside pool of the Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT) database consisting of around 1800 patients with HF who had previously consented to be approached for future research. We also identified patients from the Scottish Primary Care Research Network (SPCRN), The Scottish Health Research Register (SHARE), Generation Scotland Database, Scottish Diabetes Research Network and Wellcome Study Database. Besides identifying potential patients from various databases, we also performed opportunistic recruitment from cardiovascular and HF clinics as well as from the cardiac rehabilitation program conducted in Ninewells Hospital.

All patients who were considered suitable for the trial were provided with a patient information leaflet (Appendix 9.1) at least 24 hours prior to giving informed consent. At the first visit they were counselled by the principal investigator before an informed consent was taken (Appendix 9.2).

2.6 RANDOMISATION

After successful recruitment into the trial, patients were randomised to either dapagliflozin 10 mg or matching placebo in a double-blind fashion. The trial medication (dapagliflozin or placebo) was prepared, packaged and labelled by our onsite clinical trials pharmaceutical manufacturer. Randomisation was carried out by our dedicated clinical trials pharmacy using block randomisation. They used a validated randomisation program and securely backed-up both the randomisation seed and the treatment allocation. The allocation key was always available in the onsite 24-hour emergency unblinding facility in the Clinical Trials Pharmacy in Ninewells Hospital.

2.7 STUDY VISITS

At the screening visit an initial medical history and clinical examination was performed following informed consent. Patients had blood taken for safety analysis and vital signs recorded to confirm eligibility prior to enrolment. Patients who met the inclusion criteria and had no exclusions identified returned for a CMR scan at the Clinical Research Centre, Ninewells Hospital, Dundee within 4 weeks of the planned baseline (randomization) visit.

At the randomization visit patients performed a six-minute walk test (6MWT), quality of life questionnaire, vital signs assessment, body composition analysis (BCA) and cardio-pulmonary exercise test (CPET) measurements taken. During this visit, patients were also randomly assigned to either dapagliflozin 10 mg or matching placebo. The first dose was administered at this visit and patients were educated on the symptoms of hypoglycaemia and given a written action plan on how to manage it in the event it occurred. Patients on insulin had their total daily dose reduced by 10 % and given a 2-week glucose monitoring chart with written instructions to self-manage their insulin doses. (Appendix 9.3)

Patients returned 2 weeks later for a short safety visit where safety blood investigations (eg full blood count, renal and hepatic function tests and NT-proBNP) were performed, adverse events and vital signs were also monitored. Patients who were on insulin had their self-monitoring charts reviewed and further dose adjustments made if necessary.

This was followed by 2 more visits on months 2 and 6 with the same agenda. Three telephone visits were included in the trial follow-up schedule, at week 4, months 4 and 9. These telephone calls enabled the research team to follow up on changes in concomitant medications, adverse events and to remind the participant of study drug compliance. Compliance to study drug was determined by pill counting, carried out by the pharmacy team who report back to trial team after each returned batch.

Trial patients continued all their usual medications, which remained unchanged throughout unless clinically indicated. If any titration of a participant's other medications (eg anti-diabetic agents or diuretic agents) were indicated, changes were done in consultation with their general practitioners, and the changes recorded for analysis.

At the end of the 1-year study period, patients returned for a repeat assessment of the 6MWT, quality of life measures, BCA, CPET and CMR. Table 6 shows a summary of all visits and procedures in tabular format.

VISIT	Visit 1 Screening	Visit 2 Baseline/ Randomisation	Visit 3	Visit 4 Tele call	Visit 5	Visit 6 Tele call	Visit 7	Visit 8 Tele call	Visit 9 Final Visit	Early discontinuation visit**
	Up to 4 weeks pre visit 2	Day 0	2 week (+/- 3 days)	4 week (+/- 3 days)	Month 2 (+/- 1 week)	Month 4 (+/- 1 week)	Month 6 (+/- 1 week)	Month 9 (+/- 1 week)	Month 12 (+/- 2 weeks)	As required
Informed Consent	X									
Check Inc/Exo Criteria	X									
Medical History & Family History	X									
Clinical Examination	X									
Demographics	X									
Vital Signs	X	X	X		X		X		X	X
Safety Blood Tests & BNP	X ^A	X ^A	X ^B		X ^B		X ^B		X ^A	X ^A
Research Bloods		X							X	X
Urine pregnancy testing***	X	X	X		X		X		X	X
Genetic blood sample (if consented)		X								
MRI#		X							X	X
Bioelectrical Impedance Analysis		X	X		X		X		X	X
CPET		X							X	X
6 Min Walk Test		X							X	X
QoL Questionnaires		X							X	X
Dispense Study medication		X	X		X		X			
Adverse Event Assessment		X	X	X	X	X	X	X	X	X
Record or Review Con Meds	X	X	X	X	X	X	X	X	X	X
Check and remind on drug compliance			X	X	X	X	X	X	X	X

Table 6. Trial visits and procedures performed

#:- Screening MRI to be done only if echo criteria fulfilled. Note MRI can be done (+/- 2 weeks of scheduled visits 2 and 9 date)

Safety blood tests= X^A = U&Es, LFTs, FBC, glucose, HB A1C, BNP : X^B= U&Es, LFTs , FBC, glucose, BNP only.

** Early discontinuation visit: All tests to be done only where participant agrees. ***= Urine pregnancy testing on females of childbearing potential or who do not abstain from sex or use effective contraception.

2.8 PHARMACOVIGILANCE

The REFORM trial was conducted in accordance to the Helsinki Declaration and the principles of Good Clinical Practice (GCP). The trial was monitored at regular intervals by the Tayside Clinical Trials Unit to ensure compliance to GCP.

We ensured there was regular contact with all patients, at shorter intervals initially then gradually increased. At each visit the clinical trial team emphasized treatment compliance and enquired about any potential adverse event. If there was concern about a potential side effect or adverse event, (defined below) this was escalated to the principal investigator for further assessment. If an adverse event had occurred details of the event was recorded including date(s) of the event(s), causality, severity, action taken and date of resolution. If the event was deemed as a serious adverse event, it was reported to the Tayside Pharmacovigilance team within 24 hours.

An adverse event (AE) is defined as any untoward medical event affecting a clinical trial participant. Each initial AE will be considered for severity, causality or expectedness and may be reclassified as a serious event or reaction based on prevailing circumstances. An adverse reaction (AR) is where it is suspected that an AE has been caused by a reaction to a trial drug.

A serious adverse event (SAE), serious adverse reaction (SAR) or suspected unexpected serious adverse reaction (SUSAR) is any AE, AR or UAR that at any dose:

- results in death
- is life threatening
- requires hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- or is otherwise considered serious

2.9 OUTCOME MEASURES

2.9.1 Cardiac Magnetic Resonance Imaging

CMR was performed at randomisation and final visits only. Prior to each scan MRI safety was established for every patient by the principal investigator, then re-confirmed by the research imaging team just prior to the study as per the NHS Tayside Radiology Safety Procedures.

All imaging was performed on a 3 Tesla Magnetom Trio/PrismaFIT (Siemens, Erlangen, Germany) scanner using body array cardiac and spine matrix radiofrequency coils. In brief, the examination consisted of localizer scans followed by retrospectively gated 2D CINE segmented true fast imaging with steady state precession sequences (TrueFISP) in the vertical long axis (2 chamber) and horizontal long axis (4 chamber) orientations. This was followed by a stack of short axis plane acquisitions (using the same pulse sequence) acquired sequentially from the atrio-ventricular ring to the apex of the left ventricle. Segmentation analysis of the LV images was performed by a single blinded observer (Dr. Ify Mordi) using Argus software on a multi-modality work platform (Siemens). Contours were placed around the endocardial and epicardial borders of the LV myocardium on all short axis images acquired at end diastole and end systole. Careful segmentation rules were followed where the papillary muscles and trabeculae were assigned to the blood pool wherever possible.

2.9.2 Safety blood tests

All patients underwent a battery of routine blood investigations at every in-person visit. These tests included a full blood count, urea and electrolytes, liver function test, HbA1c (except visit 3), fasting blood sugar and NT-proBNP. These investigations (except NT-proBNP – see below) were performed by the NHS Tayside Blood Sciences Department in Ninewells Hospital according to their established procedures and protocols. The results were reviewed by the principal investigator and any action that was required (e.g. dose reduction of loop diuretic due to renal impairment) was done in consultation with the patient's GP.

2.9.3 Cardiopulmonary exercise testing

Patients performed a graded maximal bicycle exercise test with expired gas analysis using the Innocor System (Innocor, Innovision A/S, Odense, Denmark) which allows determination of peak $\dot{V}O_2$ and other exercise parameters. The CPET was performed at randomisation and final visits only. Peak oxygen uptake was defined as the highest value of oxygen uptake achieved in the final 20 seconds of exercise. As many patients with HF are unable to perform maximal exercise, and oxygen requirements for daily activities rarely approach maximal levels, a submaximal derived exercise variable of the slope of the ratio of minute ventilation ($\dot{V}E$) to carbon dioxide production ($\dot{V}E/\dot{V}CO_2$ slope) was included in results and analysis.

Due to unavoidable work and time restrictions, the principal investigator was unable to conduct final-visit CPET tests for a number of patients. This task was delegated to trained research nurses who received individual direction and training on how to conduct a CPET from the principal investigator before being delegated to carry out the test.

2.9.4 Six-minute walk test

The 6MWT is a submaximal test of an individual's functional status exercise capacity. Although correlation with NYHA classification and long-term risk stratification is poor,(179,180) it is an objective and simple tool to assess change in exercise capacity in HF.

All patients underwent 6MWT testing at the beginning and end of the trial. The test was conducted on a flat surface with a 25-meter track marked along the laboratory corridor. They were briefed on how to perform the test and were not encouraged while performing the test by the person conducting the test. Vital signs including pulse oximetry were recorded prior to and just after completing the test as a safety parameter which were not analysed.

2.9.5 Body composition analysis

As SGLT2-inhibition results in both diuresis and weight loss, we were keen to identify the degree of change in total body water and overall fat composition using a simple and non-invasive method. Measuring the bioelectrical impedance allows the estimation of total body water and fat content based on the principle that electrical current flows easily in water (with electrolytes) and is resisted by fat tissue.(181)

All patients underwent body composition analysis at every in-person visit using the TANITA BC-420-MA (Tanita Corp. Japan) machine. All patients removed their footwear (to allow direct contact with the footplates) and a standard clothes weight of 1 kg was automatically subtracted from their measured weight by the software in the machine. In the randomization and final visits, the measurements were taken before exercise tests were performed.

2.9.6 Quality of life questionnaires

Quality of life (QoL) questionnaires were conducted at the beginning and end of the study period. General and disease specific QoL measures were evaluated using the 36-item Short Form Survey (SF-36) and the Minnesota Living with Heart Failure (MLHF) questionnaires respectively.

The SF-36 had 36 questions which were then scaled and scored to provide insight into eight health domains namely, physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or

emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions. Each domain has its own mean (norm) and ranges from 0 to 100 points, higher values indicating better outcomes.(182) These domains can be grouped into two larger clusters of the Physical Component Summary (PCS) and the Mental Component Summary (MCS). As QoL is a secondary outcome of the trial, we used the two broad clusters to measure QoL. The use of the SF-36 in patients with cardiovascular disease has been previously validated.(183)

The MLHF questionnaire is one of the most widely used QoL questionnaires in patients with HF both in clinical practice and research.(184) It comprises 21 questions on a six-point Likert scale. It provides an overall score ranging from 0 to 105; with lower values indicating better QoL. Its use in the clinical and research context in patients with HF has been validated numerous times.(184,185)

The SF-36 and MLHF questionnaires have been included in Appendices 9.4 and 9.5 respectively.

2.9.7 N-Terminal Pro B-type Natriuretic Peptide

NT-proBNP has been validated and is recommended for the diagnosis of HF by international guideline committees.(23) It has also been suggested as a possible objective measure of longitudinal disease activity and been used in the follow-up of patients with chronic HF.(186)

We measured NT-proBNP concentration at every in-person visit for two reasons; firstly, as a safety measure to ensure patients were not deteriorating and required urgent intervention, secondly as a secondary outcome measure to determine if the use of an SGLT2-inhibitor (and the potential improvement in LV remodelling) resulted in lower concentrations of NT-proBNP.

Blood samples were collected by the principal investigator (or delegate) and spun immediately. Serum samples were then analysed by the University of Dundee Core Laboratory using multi array ELISA (Meso Scale Discovery, Mesoscale Diagnostics,USA).

2.9.8 Beta-hydroxybutyrate

Beta-hydroxybutyrate (BHB) is a ketone body. As discussed in detail in the previous section, SGLT2-inhibition shifts metabolism towards ketogenesis as a result of increased lipolysis and reduced insulin:glucagon ratio. This effect has been consistently documented in the T2DM population but not in the HF population till date. We also aimed to determine if an increase in BHB was associated with LV reverse remodelling as there have been suggestions that ketone bodies may be a more efficient fuel substrate for failing hearts.(62) Although only positron emission tomography or MRI-isotope tracer studies can conclusively determine changes in myocardial oxygen consumption and fuel energetics, we felt demonstrating a link between BHB and remodelling was an important hypothesis generating surrogate.

BHB levels were measured at randomisation and final visits only. Blood samples were centrifuged and serum stored for later batch analysis at the end of the trial by the University of Dundee Core Laboratory using ELISA (Varioskan Flash, Thermo-Fisher Scientific,USA).

2.10 DATA ENTRY & MANAGEMENT

Data were collected by the principal investigator or delegate identified in the delegation log with specific training in data collection. The data collected were initially noted on paper case report forms (CRF) which were stored in a secure filing cabinet in a locked room on site. Access to the CRF was only available to the principal investigator.

The data were then transcribed into Microsoft Excel (Microsoft Corp. USA) by the principal investigator or delegate in batches. Each update of the main Excel database was saved as a new file with the date of data entry as part of the filename. The database (and all other trial-related data) was stored and encrypted on the University of Dundee's cloud computing software collaboration with Box (Box Inc. USA). The data were backed up as per the university and Box Inc.'s policies. Once all data were collected, data verification and validation were performed by the principal investigator and the final database locked.

MRI data were anonymised by the research imaging team and stored locally for interpretation and analysis. A blinded observer (Dr. Ify Mordi) then analysed the data on site and transcribed the relevant research findings on to a CMR-outcomes database on Microsoft Excel. This database was then merged onto the main database just prior to data validation and database lock. All non-anonymised CMR images were stored on NHS Tayside Picture Archiving and Communication Systems (PACS) that are accessible across Scotland. These images were reviewed by Professor Graeme Houston (or delegate) to confirm

that there were no clinically actionable findings and a blinded summary report was sent to the principal investigator for record keeping.

2.11 STATISTICAL ANALYSIS

2.11.1 General Considerations

All continuous variables were summarised using the following descriptive statistics: n (non-missing sample size), number of missing records, mean, standard deviation, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels were reported for all categorical measures. In general all data were listed, sorted by subject and treatment and where appropriate by visit number within subject.

All summary tables were structured with a column for each treatment in the order and an additional column for the total population relevant to that table/treatment, including any missing observations. Student's t test (or Mann-Whitney test for non-parametric distributions) and Chi-squared for categorical variables was used to test statistical difference between groups at baseline.

Primary analysis was performed on a per-protocol basis with sensitivity analysis on an intention to treat basis. Missing data were imputed using the last observation carried forward principle.

2.11.2 Efficacy analysis

Data for continuous outcome measures were assessed for normality prior to analysis. Transformations of the outcome variables were used where necessary if not normally distributed.

If data were normally distributed, outcome measures were assessed by multivariable linear regression, controlling for baseline values, age, sex and renal function. Sensitivity analysis was also performed using models correcting for baseline NT-proBNP in view of the unexpected difference between groups at baseline. Categorical outcomes were analyzed using Pearson's chi-square. Where data were not normally distributed and could not be transformed into a normal distribution, it was analysed using non-parametric methods in addition to multiple linear regression.

Two patients were excluded from weight analysis; one in dapagliflozin arm had unexplained excessive weight gain of 9kg and one in placebo arm with 15.4kg weight loss following intensive gym program that was not informed to the research team. Both were large outliers. One patient, in the placebo arm, was excluded from all CMR-derived analysis because baseline CMR images were of too poor quality.

2.11.3 Safety analysis

An all-participant analysis was performed for all safety variables. Adverse events (AE) were coded using the Medical Dictionary for Regulatory Activities (MedDRA)(Version 21.1 English September 2018) coding system. In cases with more than one diagnosis present in the AE description, the AE was split with all the descriptors kept the same for all diagnosis. Subjects were analysed for total number of events (including recurrences) for a particular type of AE.

2.11.4 Technical details

P-values that were more than 0.001 were reported to 3 decimal places, while p-values less than 0.001 were reported as “<0.001”. The mean, standard deviation, and any other statistics other than quantiles, were reported to one decimal place. Quantiles, such as median, or minimum and maximum used the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) were reported to 2 decimal places. All analyses were performed using R version 3.4.3 for windows (R Foundation for Statistical Computing,Vienna,Austria) by the principal investigator.

3. RESULTS

3.1 STUDY RECRUITMENT

Over a recruitment period between May 2015 and August 2016 a total of 85 individuals were invited to participate in the trial. Sixty-two provided informed consent but only 56 were recruited as 3 individuals were unable to undergo CMR (1 historic metal eye injury and 2 with BMI>48kg/m² which exceeded the limitations of the scanner), 2 individuals failed pre-randomization blood tests (eGFR <45ml/min/1.73m²), and 1 individual had severe aortic regurgitation requiring intervention. Approximately 10% more than the required 52 patients were recruited to allow for dropouts during the trial as we were studying a high-risk population. (Figure 13)

Of the 56 patients recruited, 28 were assigned to the dapagliflozin and placebo arms each. Seven patients did not complete the trial; 2 in the dapagliflozin arm (1 new diagnosis of metastatic lung cancer and 1 withdrawal of consent) and 5 in the placebo arm (1 fatal myocardial infarction, 2 sudden cardiac death, 1 relapse of previously treated cholangiocarcinoma, 1 withdrawal of consent). Of 23 patients in the placebo arm who completed the trial per-protocol, CMR images for one was excluded from analysis because of poor quality at baseline however, the non CMR data points were included in analysis. There were no instances of withdrawal from the trial due to emergency unblinding throughout the study period.

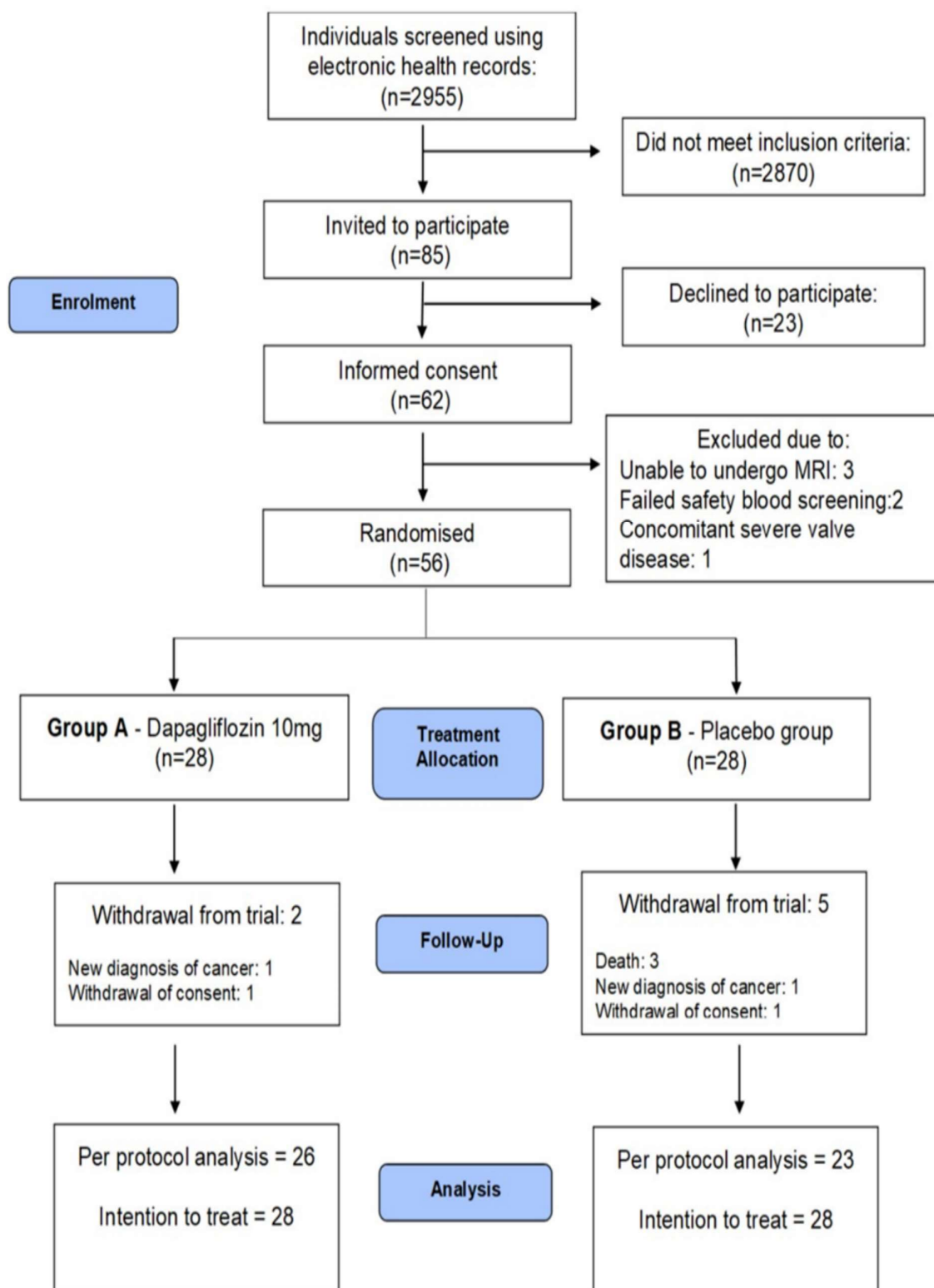


Figure 13. REFORM Trial CONSORT Diagram

3.2 BASELINE CHARACTERISTICS

The average age of the study population was 67.1 years with a male majority of 66.1% which is common in trials of cardiovascular disease. 87.5% of the cohort were in NYHA functional class I or II. The commonest aetiology for HF was ischemic heart disease, with mean CMR-derived LVEF of 45.5%.

The majority of patients were on evidence-based HF medications including ACE-I / ARB, beta-blockers and loop diuretics. Only 41.1% of patients were on mineralocorticoid receptor antagonists reflecting the mild severity of LVSD in this cohort.

With regard to T2DM therapy, just over half the cohort were on metformin and 28.6% required add-on insulin. The baseline mean HbA1c was 60.9 mmol/mol, and the mean estimated glomerular filtration rate (eGFR) was 72.0 ml/min/1.73 m².

The average duration of HF and T2DM were 6.2 and 8.8 years respectively. The commonest other co-morbidities were that of hypertension and previous myocardial infarction. The prevalence of atrial fibrillation in the REFORM cohort was 39.3% which was higher than the usual prevalence of 29.0% in UK patients with HF between 60-69 years old.(187)

Both groups were comparable for age, gender and NYHA functional class as well as the majority of other baseline parameters. There were, however, differences in the baseline measurements of NT-proBNP, and urinary albumin:creatinine ratio between the groups, with the placebo group having

significantly higher median values (Table 10). It is important to note that these values were heavily skewed, and despite this difference, there was no difference in the NYHA functional class, BP, renal function, LV volumes and LVEF between groups at baseline.

Parameter	Overall (n=56)		Dapagliflozin (n=28)		Placebo (n=28)		p-value
	Mean /	SD /	Mean /	SD /	Mean /	SD /	
	(Med)	(IQR)	(Med)	(IQR)	(Med)	(IQR)	
Demographics:							
Age (years)	67.1	6.9	66.9	7.0	67.4	6.8	0.788
Male gender	37	66.1%	18	64.3%	19	67.9%	0.054
Weight (kg)	91.5	18.6	92.9	18.1	90.2	19.3	0.587
BMI (kg/m ²)	32.5	5.3	33.0	5.5	32.0	5.2	0.495
Functional Class:							
NYHA I	25	44.6%	12	42.9%	13	46.4%	0.794
NYHA II	24	42.9%	13	46.4%	11	39.3%	0.546
NYHA III	7	12.5%	3	10.7%	4	14.3%	0.245
Blood pressure / Heart Rate:							
SBP (mmHg)	133.9	17.1	135.0	15.4	132.8	18.8	0.627
DBP (mmHg)	72.7	10.0	74.0	7.1	71.4	12.2	0.328
MAP (mmHg)	93.1	10.4	94.4	7.3	91.9	12.8	0.373
HR (bpm)	76.0	12.8	77.6	14.0	74.4	11.6	0.354
Duration of disease:							
Heart failure (years)	6.24	6.49	6.64	6.07	5.83	6.99	0.649
Type 2 diabetes (years)	8.77	6.26	8.41	6.45	9.14	6.17	0.672

Table 7. Baseline demographic data

Abbreviations: Med=Median; SD=Standard deviation; IQR=Interquartile range; CI=Confidence interval; kg= kilograms; BMI=body mass index;

NYHA=New York Heart Association functional classification for heart failure;
SBP= systolic blood pressure; DBP=Diastolic blood pressure; MAP=Mean
arterial pressure; HR=Heart Rate; bpm=beats per minute.

Parameter	Overall (n=56)		Dapagliflozin (n=28)		Placebo (n=28)		p-value
	Mean / (Med)	SD / (IQR)	Mean / (Med)	SD / (IQR)	Mean / (Med)	SD / (IQR)	
Etiology of heart failure:							
IHD	30	53.6%	15	53.6%	15	53.6%	0.845
DCM	13	23.2%	7	25.0%	6	21.4%	0.456
Other	11	19.6%	5	17.9%	6	21.4%	0.124
Unknown	2	3.6%	1	3.6%	1	3.6%	1.000
Co-morbidities:							
Hypertension	40	71.4%	22	78.6%	18	64.3%	0.485
AF	22	39.3%	11	39.3%	11	39.3%	0.998
PAD	9	16.1%	5	17.9%	4	14.3%	0.842
Stroke / TIA	10	17.9%	3	10.7%	7	25.0%	0.060
MI	29	51.8%	14	50.0%	15	53.6%	0.475
Medications:							
Loop diuretic dose (mg)*	49.8	22.0	52.9	20.5	46.8	23.4	0.307
ACE-I/ARB	50	89.3%	25	89.3%	25	89.3%	0.985
Beta blockers	46	82.1%	24	85.7%	22	78.6%	0.877
MRA	23	41.1%	13	46.4%	10	35.7%	0.898
Metformin	31	55.4%	17	60.7%	14	50.0%	0.978
Other OHA	22	39.3%	13	46.4%	9	32.1%	0.413
Insulin	16	28.6%	6	21.4%	10	35.7%	0.657

Table 8. Aetiology of HF, co-morbidities and concurrent medications

Abbreviations: IHD=Ischemic heart disease; DCM=Dilated cardiomyopathy; AF=Atrial fibrillation; PAD=Peripheral arterial disease; TIA=Transient ischemic attack; MI=Myocardial infarction; ACE-I=Angiotensin converting enzyme inhibitor; ARB=Angiotensin receptor blocker; MRA=Mineralocorticoid receptor antagonist; OHA=Oral hypoglycemic agent.

* Bumetanide dose converted to equivalent furosemide dose (1mg bumetanide = 40mg furosemide)

Parameter	Overall (n=55)*		Dapagliflozin (n=28)		Placebo (n=27)*		p-value
	Mean /	SD /	Mean /	SD /	Mean /	SD /	
	(Med)	(IQR)	(Med)	(IQR)	(Med)	(IQR)	
Cardiac MRI Parameters:							
LVEDV (ml)	180.2	61.0	172.4	47.7	188.3	72.4	0.346
Indexed LVEDV (ml/m²)	90.3	30.9	85.9	24.1	95.0	36.8	0.294
LVESV (ml)	102.7	50.5	99.2	40.7	106.4	59.6	0.604
Indexed LVESV (ml/m²)	51.6	26.2	49.4	21.3	54.1	30.9	0.520
LV Mass Index (g/m²)	71.5	17.7	69.5	16.3	73.7	19.3	0.398
LVEF (%)	45.5	12.0	44.5	12.4	46.5	11.7	0.526
LV Stroke Vol (ml)	38.7	10.4	36.6	10.4	41.0	10.2	0.126
Indexed LA Volume(ml/m²)†	50.0	18.9	49.0	18.8	51.0	19.3	0.699

Table 9. Cardiac Magnetic Resonance Imaging parameters at baseline

Abbreviations: LVEDV=Left ventricular end diastolic volume; LVESV=Left ventricular end systolic volume; LVEF=Left ventricular ejection fraction; LV=Left ventricular; LA=Left atrial

* = Unable to analyse baseline images of one participant in placebo group due to very poor image quality

† = Unable to analyze baseline LA volume in 5 patients due to poor image quality (overall n= 51, dapagliflozin n=26, placebo n=25)

Parameter	Overall (n=56)		Dapagliflozin (n=28)		Placebo (n=28)		p-value
	Mean /	SD /	Mean /	SD /	Mean /	SD /	
	(Med)	(IQR)	(Med)	(IQR)	(Med)	(IQR)	
Biochemistry:							
Hb (g/dL)	13.5	1.6	13.2	1.3	13.8	1.5	0.294
Hct (%)	40.9	4.7	40.4	3.9	41.4	5.4	0.405
HbA1c (mmol/mol)	60.9	17.1	63.0	17.8	58.6	16.4	0.339
Creatinine (μmol/L)	88.2	19.9	92.0	19.9	84.4	19.5	0.151
eGFR (ml/min/1.73m²)	72.0	19.2	67.7	16.4	76.2	21.0	0.254
BHB (mmol/L)	0.2	0.1	0.2	0.1	0.2	0.1	0.865
Biomarkers:							
NT-ProBNP (pg/ml)	(1781.6)	(4910.4)	(1567.9)	(1735.1)	(3717.3)	(5846.6)	0.028
hsCRP (mg/L)	(3.3)	(6.6)	(3.3)	(4.8)	(3.8)	(7.4)	0.873
Oxi-LDL (u/ml)	54.3	19.2	59.2	19.8	49.4	17.6	0.054
Urinary measurements:							
Urine Na (mmol/L)	80.7	33.6	79.0	32.6	82.4	35.0	0.708
Urine Alb (mg/L)	(13.0)	(40.8)	(9.0)	(29.0)	(19.0)	(53.5)	0.132
Urine ACR (mg/mmol)	(2.9)	(10.2)	(1.8)	(5.4)	(4.4)	(14.0)	0.0503

Table 10. Blood and urine investigations at baseline

Abbreviations: Hb=Haemoglobin; Hct=Haematocrit; HbA1c=Haemoglobin A1c; eGFR=Estimated glomerular filtration rate; NT-proBNP=N-terminal pro B-type natriuretic peptide; hsCRP=High sensitivity C-reactive protein; Oxi-LDL=Oxidized low density lipoprotein; Na=Sodium; Alb=Albumin; ACR=Albumin:creatinine ratio

Parameter	Overall (n=56)		Dapagliflozin (n=28)		Placebo (n=28)		p-value
	Mean /	SD /	Mean /	SD /	Mean /	SD /	
	(Med)	(IQR)	(Med)	(IQR)	(Med)	(IQR)	
Cardiopulmonary exercise test parameters:							
VO ₂ Max* (ml/min)	1290.0	350.0	1290.0	350.0	1300.0	360.0	0.979
VCO ₂ Max* (ml/min)	(1200.0)	(530.0)	(1210.0)	(500.0)	(1170.0)	(540.0)	1.000
Peak Ve* (L/min)	50.7	14.7	50.2	14.1	51.3	15.6	0.782
Normalized VO ₂ Max*(ml/kg/min)	14.3	3.4	14.1	3.3	14.5	3.5	0.659
Ve/VCO ₂ slope*	37.6	6.8	36.3	6.3	38.9	7.2	0.213
Respiratory exchange ratio*	0.97	0.1	0.97	0.1	0.96	0.1	0.802
Six-minute walk test:							
Distance (m)	398.2	128.9	404.3	113.8	392.2	144.4	0.857
Quality of life questionnaires:							
SF-36 PCS	50.0	10.6	50.4	10.9	49.5	10.5	0.747
SF-36 MCS	49.5	9.9	51.2	10.6	47.9	9.1	0.226
MLHF	(24.5)	(30.0)	(22.0)	(31.0)	(25.5)	(27.8)	0.712

Table 11. Exercise capacity and quality of life measures at baseline

Abbreviations: V_{O₂} Max=Peak oxygen consumption; VCO₂ Max=Peak carbon dioxide production; Ve=minute ventilation; Ve/VCO₂= peak minute ventilation

to carbon dioxide ratio; SF-36 PCS=Physical component summary of the short form 36; SF-36 MCS=Mental component summary of the short form 36; MLHF=Minnesota living with heart failure.

*=Analysis excludes 2 patients in placebo arm who were unable to complete CPET

3.3 CARDIAC MRI FINDINGS

At the end of one year, per-protocol analysis revealed no significant difference in LV volumes of patients in both groups when adjusted for baseline ventricular volumes, age, sex and renal function. Both LVEDV and LVESV were reduced in dapagliflozin and placebo groups (LVEDV: dapagliflozin -6.9 ± 35.9 ml vs. placebo -12.8 ± 41.4 ml, adjusted treatment effect $+4.15$ ml; 95% CI -18.52 - 26.83 , $p=0.714$; LVESV: dapagliflozin -7.0 ± 26.7 ml vs. placebo -8.1 ± 36.5 ml, adjusted treatment effect $+0.96$ ml; 95% CI -17.07 - 19.00 , $p=0.915$). There was no significant difference in LVEF (dapagliflozin $+2.1 \pm 5.9\%$ vs. placebo $-0.3 \pm 8.7\%$, adjusted treatment effect $+1.98\%$; 95% CI -2.18 - 6.14 , $p=0.342$) or LVMI (dapagliflozin $+3.7 \pm 10.1$ g/m² vs. placebo $+3.6 \pm 8.9$ g/m², adjusted treatment effect -0.05 g/m²; 95% CI -5.98 - 5.89 , $p=0.970$)

Sensitivity analysis was performed as per the prespecified analytical protocol using intention to treat; the observed effects were unchanged. A further post-hoc sensitivity analysis was performed using the per-protocol dataset controlling for baseline NT-proBNP as this was unexpectedly different between groups at baseline. Once again, there was no change in the treatment effects seen.

Outcome	Dapagliflozin (n=26)		Placebo (n=22)*		Adjusted treatment effect (95% CI)	p value
	Mean	SD	Mean	SD		
LVEDV (ml)	-6.9	35.9	-12.8	41.4	4.15 (-18.52 to 26.83)	0.714
Indexed LVEDV (ml/m²)	-3.4	15.7	-6.6	20.5	1.93 (-8.57 to 12.43)	0.713
LVESV (ml)	-7.0	26.7	-8.1	36.5	0.96 (-17.07 to 19.00)	0.915
Indexed LVESV (ml/m²)	-3.3	12.3	-4.1	18.1	0.51 (-10.97 to 11.99)	0.929
LVEF (%)	2.1	5.9	-0.3	8.7	1.98 (-2.18 to 6.14)	0.342
LV Indexed Mass (g/m²)	3.7	10.1	3.6	8.9	-0.05 (-5.98 to 5.89)	0.970

LV Stroke Vol (ml)	-0.3	6.5	-2.5	6.6	1.50 (-2.27 to 5.27)	0.426
Indexed LA Volume (ml/m²)[†]	-2.7	11.8	-3.5	14.9	-2.45 (-10.62 to 5.71)	0.546

Table 12. Cardiac MRI outcomes

Abbreviations: SD=Standard deviation; CI=Confidence interval; LVEDV=Left ventricular end diastolic volume; LVESV=Left ventricular end systolic volume; LVEF=Left ventricular ejection fraction; LV=Left ventricular

* =1 patient in placebo group excluded from analysis due to uninterpretable baseline CMR films – dapagliflozin (n=26), placebo (n=22)

† = 6 patients excluded due to poor baseline and or final image quality (dapagliflozin n=23, placebo n=19)

3.4 METABOLIC AND HAEMODYNAMIC EFFECTS

After 1 year of treatment, dapagliflozin caused a reduction in body weight that approached statistical significance (dapagliflozin -1.9 ± 3.8 kg vs. placebo -0.9 ± 4.7 kg, adjusted treatment effect -1.97 kg; 95% CI -3.99 - 0.05 , $p=0.056$). However, when the full dataset was analyzed (to include the two large outliers) the adjusted treatment effect was -0.89 kg; 95% CI -3.31 - 1.53 , $p=0.464$. There was a significant reduction in diastolic blood pressure (dapagliflozin -0.1 ± 7.5 mmHg vs. placebo $+6.1 \pm 11.1$ mmHg, adjusted treatment effect -6.58 mmHg; 95% CI -11.93 - -1.23 , $p=0.017$). Systolic blood pressure was numerically lower in the dapagliflozin arm (dapagliflozin -3.5 ± 19.3 mmHg vs. placebo $+2.4 \pm 19.4$ mmHg, adjusted treatment effect -4.80 mmHg; 95% CI -16.04 - 6.44 , $p=0.39$). Despite reduction in blood pressure there was no increase in heart rate (dapagliflozin -3.0 ± 9.3 beats/min vs. placebo -2.0 ± 11.6 beats/min, adjusted treatment effect $+0.040$ beats/min; 95% CI -5.67 - 6.48 , $p=0.894$).

Outcome	Dapagliflozin (n=26)		Placebo (n=23)		Adjusted treatment effect (95% CI)	p value
	Mean	SD	Mean	SD		
Weight [†] (kg)	-1.9	3.8	-0.9	4.7	-1.97 (-3.99 to 0.05)	0.056

SBP (mmHg)	-3.5	19.3	2.4	19.4	-4.80 (-16.04 to 6.44)	0.394
DBP (mmHg)	-0.1	7.5	6.1	11.1	-6.58 (-11.93 to -1.23)	0.017
MAP (mmHg)	-1.2	9.9	4.9	13.1	-5.99 (-12.65 to 0.68)	0.077
Heart rate (beats/min)	-3.0	9.3	-2.0	11.6	0.40 (-5.67 to 6.48)	0.894

Table 13. Metabolic and haemodynamic outcomes

Abbreviations: SBP=Systolic blood pressure; DBP=Diastolic blood pressure;
MAP= Mean arterial pressure

†=analysis excludes 1 patient from each arm due to excessive weight gain and loss (see 2.11.2 Efficacy Analysis) –dapagliflozin (n=25), placebo (n=22);

3.5 BLOOD AND BIOCHEMISTRY

Patients in the dapagliflozin arm saw a significant increase in haemoglobin (dapagliflozin $+1.2 \pm 1.1$ g/dL vs. placebo -0.1 ± 0.8 g/dL, adjusted treatment effect $+1.16$ g/dL; 95% CI 0.60-1.74, $p < 0.001$) and haematocrit (dapagliflozin $+4.0 \pm 3.0\%$ vs. placebo $0.0 \pm 3.0\%$, adjusted treatment effect $+3.59\%$; 95% CI 1.87-5.31, $p < 0.001$). Dapagliflozin also caused a modest but significant increase in BHB (dapagliflozin $+0.03 \pm 0.06$ mmol/L vs. placebo 0.00 ± 0.06 mmol/L, adjusted treatment effect $+0.04$ mmol/L; 95% CI 0.001-0.08, $p = 0.045$). Notably, there was no significant difference in log NT-proBNP at the end of the trial, (dapagliflozin -0.1 ± 0.8 pg/mL vs. placebo -0.2 ± 0.9 pg/mL, adjusted treatment effect 0.00 pg/mL; 95% CI -0.53 - 0.53 , $p = 0.993$) even in additional sensitivity analysis adjusting for body weight.

Unexpectedly, in the treatment arm, we also observed a significant increase in oxidized-LDL (oxi-LDL) (dapagliflozin $+2.0 \pm 12.6$ u/mL vs. placebo -5.6 ± 15.2 u/mL, adjusted treatment effect $+10.70$ u/mL; 95% CI 3.22-18.19, $p = 0.006$) and reduction in urinary sodium excretion (dapagliflozin -13.7 ± 50.6 mmol/L vs. placebo $+5.7 \pm 31.7$ mmol/L, adjusted treatment effect -16.9 mmol/L; 95% CI 37.12 - -3.34 , $p = 0.036$).

Outcome	Dapagliflozin (n=26)		Placebo (n=23)		Adjusted treatment effect (95% CI)	p value
	Mean	SD	Mean	SD		
Haemoglobin (g/dL)	1.2	1.1	-0.1	0.8	1.16 (0.60 to 1.74)	<0.001
Haematocrit (%)	4.0	3.0	0.0	3.0	3.59 (1.87 to 5.31)	<0.001
BHB (mmol/L)	0.03	0.06	0.00	0.06	0.04 (0.001 to 0.08)	0.045
Oxidized-LDL (u/ml)	2.0	12.6	-5.6	15.2	10.70 (3.22 to 18.19)	0.006
Log hsCRP (mg/L)	0.0	1.1	-0.3	0.9	0.16 (-0.40 to 0.80)	0.531

Serum creatinine ($\mu\text{mol/L}$)	5.1	12.9	6.6	9.5	-2.42 (-9.20 to 4.35)	0.480
eGFR (ml/min/1.73m ²)	-1.5	12.5	-6.9	10.8	4.23 (-2.81 to 11.27)	0.232
Log NT-proBNP (pg/ml)	-0.1	0.8	-0.2	0.9	0.00 (-0.53 to 0.53)	0.993
HbA1c (mmol/mol)	-3.3	9.3	-1.4	11.4	-1.30 (-7.07 to 4.48)	0.653
Urine Na (mmol/L)	-13.7	50.6	5.7	31.7	-16.9 (-37.12 to -3.34)	0.036
Log Urine albumin (mg/L)	0.1	1.6	-0.6	1.8	0.20 (-0.85 to 1.25)	0.704
Log Urinary ACR	0.0	1.1	-0.5	0.8	0.30 (-0.33 to 0.92)	0.343

Table 14. Blood and biochemistry outcomes

Abbreviations: BHB=Beta-hydroxybutyrate; LDL=Low density lipoprotein; hsCRP=High sensitivity C-reactive protein; eGFR=Estimated glomerular filtration rate; NT-proBNP=N-terminal pro B-type natriuretic peptide; Na=Sodium; ACR=Albumin:creatinine ratio

3.6 LOOP DIURETIC THERAPY

Patients on the dapagliflozin arm required less loop diuretic therapy compared to those on placebo (dapagliflozin $-16.9 \pm 18.5\text{mg}$ vs. placebo $9.1 \pm 28.8\%$, adjusted treatment effect -28.04mg ; 95% CI -42.35 - -13.74 , $p < 0.001$) and were more likely to stop or reduce their loop diuretic dose; 50.0% vs 8.7%; $p = 0.005$.

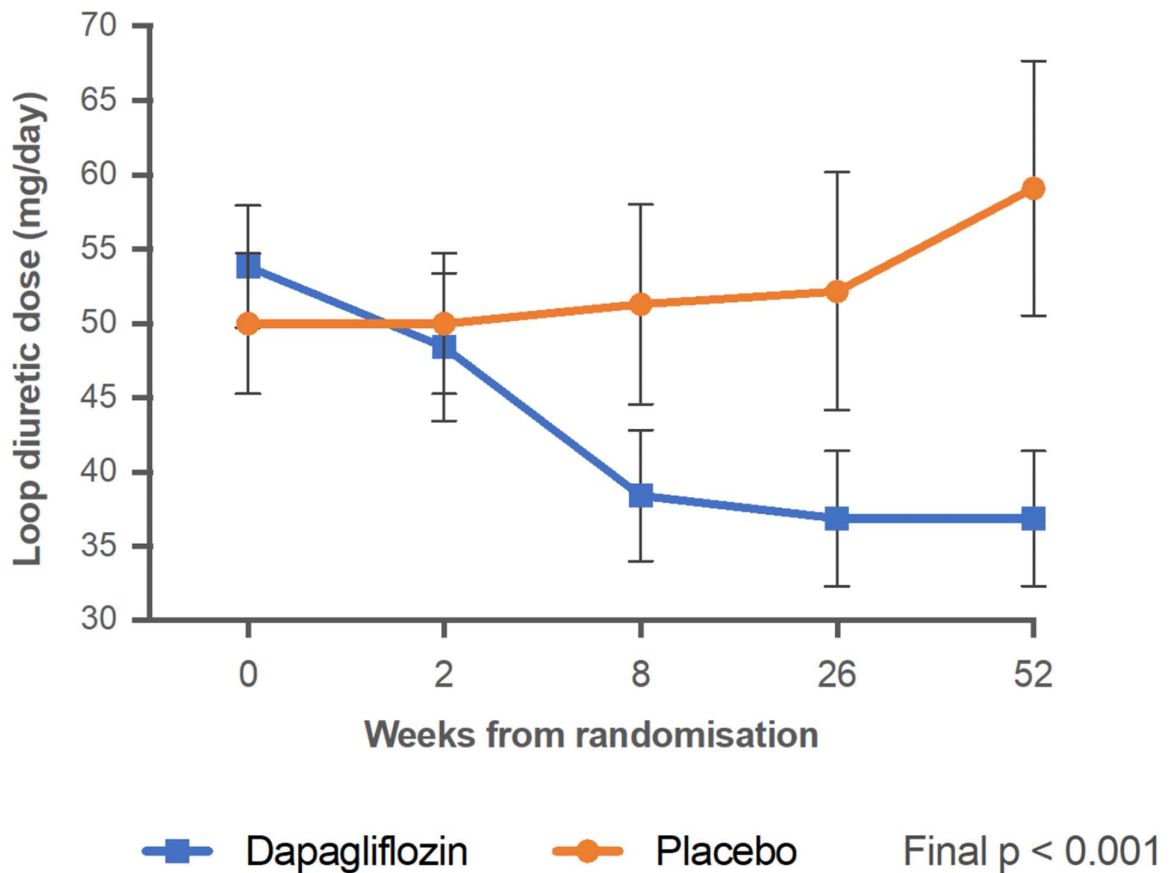


Figure 14. Loop diuretic dose trend

Note bumetanide dose converted to equivalent furosemide dose (1mg bumetanide = 40mg furosemide)

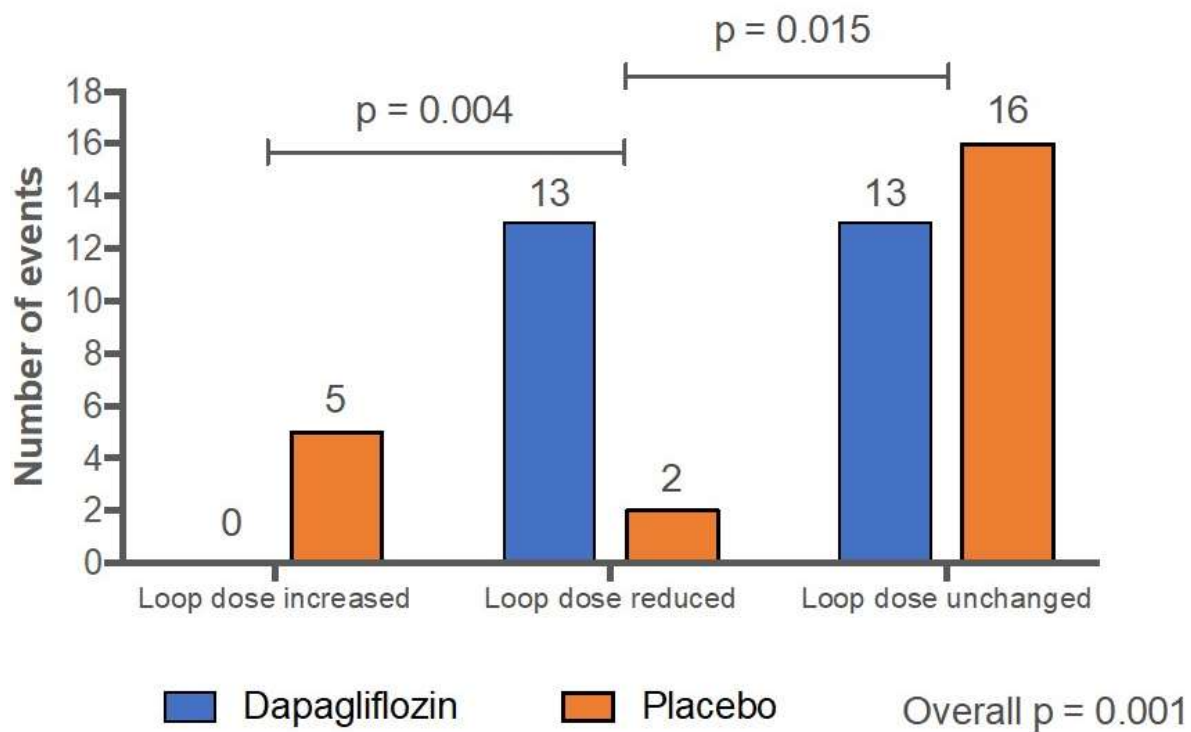


Figure 15. Changes in loop diuretic requirements

Note bumetanide dose converted to equivalent furosemide dose (1mg bumetanide = 40mg furosemide)

3.7 EXERCISE TOLERANCE AND BODY COMPOSITION

There was no significant difference between the two groups with regard to measures of exercise tolerance such as CPET parameters or 6MWT distance. Similarly, there was no difference between groups in total body fat or water content at the end of the trial.

Unfortunately, due to improper technique in the final CPET assessment (ill-fitting mask, poor adherence to exercise protocol and early termination of test), a total of 6 patients had to be excluded from final analysis. This was due to the delegation of trial data collection duties to research nurses as the principal investigator was unavailable.

Outcome	Dapagliflozin (n=26)		Placebo (n=23)		Adjusted treatment effect (95% CI)	p value
	Mean	SD	Mean	SD		
VO₂ Max* (ml/min)	-0.1	0.3	-0.1	0.4	0.02 (-0.19 to 0.24)	0.848
Log VCO₂ Max* (ml/min)	-0.1	0.3	-0.2	0.6	0.08 (-0.13 to 0.30)	0.439

Peak Ve* (L/min)	-2.1	8.9	-1.9	11.3	0.64 (-5.92 to 7.20)	0.845
Normalized VO₂ Max* (ml/kg/min)	2.8	16.5	-1.0	3.6	3.28 (-5.43 to 11.98)	0.450
Respiratory exchange ratio	-0.0	0.1	-0.0	0.1	-0.01 (-0.05 to 0.02)	0.420
Ve/VCO₂ Slope*	1.3	4.9	0.4	7.4	1.95 (-1.94 to 5.84)	0.315
6MWT distance (m)	-14.1	81.9	1.7	45.3	-20.71 (-62.19 to 20.78)	0.320
Total body fat (%)	-0.1	2.6	-0.3	2.8	0.42 (-1.06 to 1.91)	0.571
Total body water (%)	-0.2	1.4	0.0	1.6	-0.38 (-1.18 to 0.43)	0.351

Table 15. Exercise tolerance and body composition outcomes

Abbreviations: $\dot{V}O_2$ Max=Peak oxygen consumption; $\dot{V}CO_2$ Max=Peak carbon dioxide production; \dot{V}_E =minute ventilation; $\dot{V}_E/\dot{V}CO_2$ = peak minute ventilation to carbon dioxide ratio; 6MWT=Six-minute walk test

*=analysis excludes 6 patients due to incorrect CPET technique / uninterpretable data at final visit test. (dapagliflozin n=25 and placebo n=18).

3.8 QUALITY OF LIFE

There was no difference in all measures of general or specific quality of life measures using the SF-36 and MLHF questionnaires respectively.

Outcome	Dapagliflozin (n=26)		Placebo (n=23)		Adjusted treatment effect (95% CI)	p value
	Mean	SD	Mean	SD		
SF-36 PCS	0.2	6.4	-0.1	5.9	-0.06 (-3.50 to 3.37)	0.970
SF-36 MCS	-0.4	10.5	-0.1	7.3	1.33 (-3.93 to 6.59)	0.614
MLHFQ	-1.2	18.7	0.6	20.8	-1.19 (-12.08 to 9.69)	0.826

Table 16. Quality of life outcomes

Abbreviations: SF-36 PCS=Physical component summary of the short form 36; SF-36 MCS=Mental component summary of the short form 36; MLHF=Minnesota living with heart failure

3.9 ADVERSE EVENTS

There was a total of 5 deaths during the trial period; 1 in the dapagliflozin arm and 4 in the placebo arm, 3 of them CV deaths. There was a total of 4 instances of decompensated HF, one of them recurrent in a participant who was on placebo. There were 3 cases of acute coronary syndromes; 1 each of a fatal myocardial infarction, non-STEMI and unstable angina, all occurring in the placebo arm.

We observed numerically more cases of urinary tract infections / genital infections in the dapagliflozin arm compared to placebo; 5 vs 2 cases. Two patients had recurrent infections, both were on dapagliflozin. All instances of infection were treated by the GP without having to withdraw / withhold therapy.

There were significantly more instances of major worsening of renal function (defined as >20% increase in creatinine or eGFR <45 ml/min/1.73 m²) in the dapagliflozin arm.

Adverse Event Breakdown:

There were 64 recorded instances of AEs, 13 of which were SAEs. None of the SAEs were attributed to the study drug. Of the 51 AEs 17 were classified as drug-related in the dapagliflozin group, while there were 7 such events in the placebo group.

SAEs in Dapagliflozin group (3 events):

- Decompensated HF
- New diagnosis of metastatic lung cancer
- Gastroenteritis requiring hospitalisation

SAEs in Placebo group (10 events):

- Decompensated HF
- Recurrence of cholangiocarcinoma
- Gastroenteritis requiring hospitalisation
- Acute coronary syndrome (3 individuals)
- Cardiac arrest from ventricular fibrillation (2 individuals)
- Community acquired pneumonia requiring hospitalisation
- Malignant otitis externa

The following are some key adverse events of interest:

Parameter	Dapagliflozin	Placebo	p value
All cause death	1	4	0.349
Fatal MI	0	1	1.00
Sudden cardiac death	0	2	0.472
Non-CV death	1	1	1.00
Decompensated HF	1	3	0.604
Non-fatal ACS	0	2	0.472
Cancer (new / recurrence)	1	1	1.00
Other hospitalizations	1	3	0.604
Major WRF*	8	0	0.008
Minor WRF*	0	2	0.472
Symptomatic hypoglycemia	4	2	0.666

Dehydration	3	0	0.235
UTI / genital infections	5	2	0.419
Other non-serious adverse events	12	15	0.593

Table 17. Adverse events

Abbreviations: MI=Myocardial infarction; CV=Cardiovascular; HF=Heart failure; ACS=Acute coronary syndrome; WRF=Worsening renal function; UTI=Urinary tract infection

*= Major WRF was defined as >20% increase in creatinine or eGFR <45 ml/min/1.73 m². Minor WRF was defined as a sustained increase in creatinine that was <20% from baseline and maintaining eGFR >45 ml/min/1.73 m².

3.10 EXPLORATORY ANALYSIS

The inclusion criteria for the REFORM trial stipulated that patients had to have a diagnosis of HF with echocardiographic evidence of LV systolic dysfunction defined as LVEF <45% or at least mild dysfunction on subjective assessment by the sonographer. Although it was not ideal to use a subjective assessment of LV function for inclusion, the vast majority of echocardiography reports in the Tayside region did not objectively measure LV function (e.g. by Simpson's biplane or global longitudinal strain) meaning it would have been extremely difficult to recruit patients by screening electronic health records. Pre-recruitment echocardiography would have resolved this issue but due to limited funding and staff availability, this was not an option.

This resulted in some patients with LVEF that were $\geq 45\%$ being recruited into the trial as evidenced the CMR-derived baseline LVEF range of 20.0% to 75%. Although there is an expected difference between these measurements using two very different imaging modalities, this introduced heterogeneity into the cohort and made it difficult to investigate the true effects of the drug in patients with ongoing HFrEF.

To mitigate this, we performed a post-hoc, exploratory analysis by looking for an interaction between LVEF and treatment allocation. We used an LVEF cutoff of 45% as the interaction term in the regression model, thus allowing analysis of the cohort as originally intended by the inclusion criteria. Interaction analysis is superior to simply splitting the cohort and performing subgroup analysis on those with baseline LVEF <45% because with interaction analysis

the population size is not reduced, thereby preserving the overall power of the analysis.

Table 18 and Table 19 show some key baseline and final measurements for both subgroups. We then re-ran the regression model with the interaction term of baseline LVEF for the primary outcome and relevant secondary outcome measures. The key findings of this analysis are summarized in Figure 16. Significant interactions were found only in LVEDV, LVESV and LVMI. Unexpectedly, it showed that patients with higher baseline LVEF ($\geq 45\%$) saw LV reverse remodelling while those with lower LVEF didn't. There was no interaction seen between baseline LVEF and dapagliflozin in other secondary outcomes.

We were keen to understand the reason for this differential effect and ascertain if there were any confounders that were masking the true mechanism behind this. We first tested if change in BP influenced LV remodelling - BP reduction is an established factor in inducing reverse remodelling,(188-192) and observing this would help validate our data. Indeed, we observed that SBP reduction was associated with significant reductions in LVEDV, LVESV and LVMI; there was a -1.11ml (95% CI -0.58 to -1.63, $p < 0.001$), -0.69ml (95% CI -0.24 to -1.13, $p = 0.004$) and -0.26ml (95% CI -0.12 to -0.40, $p < 0.001$) reduction respectively for every 1mmHg reduction in SBP. However, change in SBP had no effect on LVEF. These effects were independent of baseline LVEF.

Having established that reduction in SBP led to LV reverse remodelling in the entire cohort, regardless of baseline LVEF, we wanted to determine if there was a difference in the blood pressure reduction achieved between those with higher LVEF ($\geq 45\%$) and lower LVEF ($< 45\%$) at baseline. There was a greater reduction in BP achieved in the higher LVEF group by $-11.35/-5.60$ mmHg ($P_{\text{sbp}}=0.046/P_{\text{dbp}}=0.038$), adjusted for baseline BP, age, sex and baseline eGFR. Importantly, there was no interaction between baseline LVEF and treatment allocation with regard to change in BP, ($P_{\text{interaction}}=0.055$ for SBP and $P_{\text{interaction}}=0.118$ for DBP) in other words, the effect of dapagliflozin on BP was unaffected by baseline LVEF. This suggested that there was another reason behind this difference in BP reductions between the LVEF groups.

Interestingly, although both LVEF subgroups saw reductions in mean loop diuretic doses when on dapagliflozin, there was significantly greater reduction in loop diuretic dose in the lower LVEF group ($P_{\text{interaction}}=0.010$). This was very likely driven by lower baseline eGFR in the dapagliflozin arm of the lower LVEF subgroup (Table 19) which would have deteriorated further following increased diuresis caused by the trial drug. We also observed patients with any reduction in loop diuretic dose ended up with higher mean final SBP ($p=0.016$) but not DBP ($p=0.482$). We confirmed that change in loop diuretic dose alone did not result in any significant changes in measures of LV remodelling.

The implications of these exploratory, post hoc findings are discussed in detail in section 4.5.

Parameter	BASELINE LVEF < 45% (n=19)					BASELINE LVEF ≥ 45% (n=29)				
	DAPA (n=11)		PLACEBO (n=8)		*p-value	DAPA (n=15)		PLACEBO (n=14)		*p-value
	Median	IQR	Median	IQR		Median	IQR	Median	IQR	
Start LVEDV	182.9	32.4	230.1	93.5	0.216	163.4	65.2	137.0	82.4	0.541
Final LVEDV	175.8	35.5	183.6	65.8	0.934	144.7	43.6	145.1	68.3	0.861
Absolute diff	-7.1		-46.5		0.058	-18.7		8.1		0.359
Start LVESV	123.9	29.8	149.95	74.5	0.509	75.5	44.0	64.8	36.6	0.760
Final LVESV	114.6	18.6	105.6	55.7	0.680	65.8	24.8	59.1	47.5	0.793
Absolute diff	-9.3		-44.35		0.117	-9.7		-5.7		0.176
Start LVMI	72.1	15.3	84.2	16.9	0.248	69.6	18.4	64.4	24.7	0.861
Final LVMI	73.3	17.1	79.8	19.8	0.869	69.3	22.0	69.9	27.6	0.541
Absolute diff	1.2		-4.4		0.364	-0.3		5.5		0.064
Start LVEF	32.7	8.1	41.7	8.7	0.058	52.0	8.7	49.8	10.1	0.727
Final LVEF	36.3	8.6	44.9	4.4	0.026	56.4	8.6	50.0	18.0	0.285
Absolute diff	3.6		3.2		0.620	4.4		0.2		0.070

Table 18. Measures of LV remodelling split by baseline LVEF

Abbreviations: LVEDV=Left ventricular end diastolic volume; LVESV=Left ventricular end systolic volume; LVEF=Left ventricular ejection fraction; LVMI=Left ventricular indexed mass; Diff=Difference; DAPA=Dapagliflozin; IQR=Interquartile range

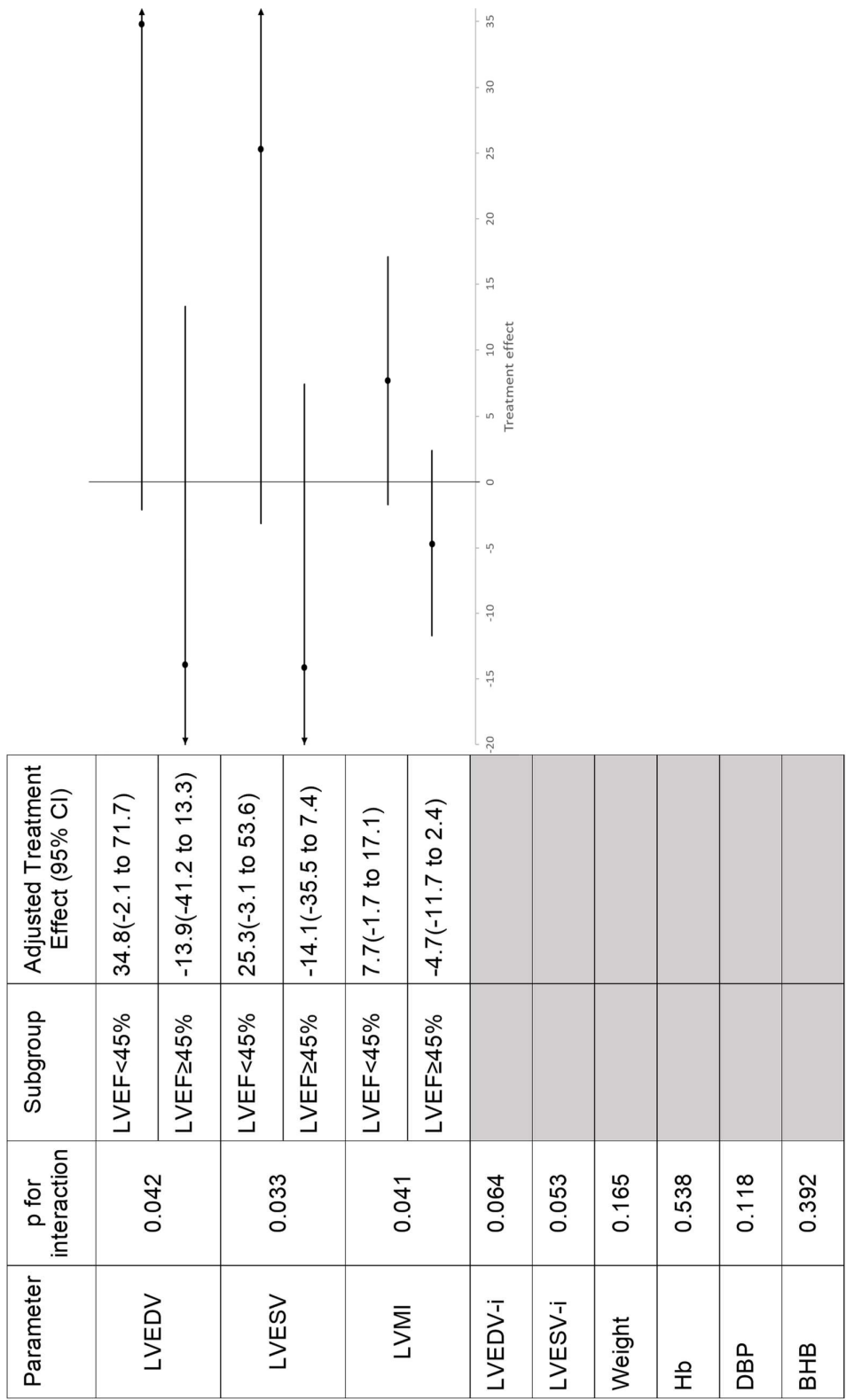
*= Test of significance used was Wilcoxon signed rank test

Parameter	BASELINE LVEF < 45% (n=19)					BASELINE LVEF ≥ 45% (n=29)				
	DAPA (n=11)		PLACEBO (n=8)		*p- value	DAPA (n=15)		PLACEBO (n=14)		*p- value
	Median	IQR	Median	IQR		Median	IQR	Median	IQR	
Start Weight	94.4	15.9	85.2	17.0	0.186	97.0	21.6	92.1	15.9	0.631
Final Weight	97.5	16.8	85.5	9.3	0.186	92.4	16.9	92.4	14.6	0.861
Absolute diff	3.1		0.3		0.680	-4.6		0.3		0.032
Start SBP	130.0	30.5	129.5	35.8	0.901	142.0	22.0	127.0	29.0	0.678
Final SBP	139.0	22.5	131.0	15.3	0.282	121.0	14.5	138.5	35.8	0.097
Absolute diff	9.0		1.5		0.710	-21		11.5		0.042
Start DBP	73.0	9.5	76.5	16.8	0.934	72.0	8.5	73.0	10.5	0.693
Final DBP	75.0	12.5	74.5	12.5	0.934	70.0	7.0	80.0	15.8	0.006
Absolute diff	2		-2		0.281	-2		7		0.019
Start eGFR	60.4	22.0	76.5	13.0	0.039	68.4	33.6	67.1	36.9	0.485
Final eGFR	56.6	27.6	70.6	13.4	0.186	69.5	27.4	67.9	29.4	0.695
Absolute diff	-3.8		-5.9		0.283	1.1		0.8		0.570
Start Loop dose	60.0	40.0	40.0	12.5	0.083	40.0	30.0	40.0	40.0	0.602
Final Loop dose	40.0	0.0	60.0	40.0	0.127	40.0	20.0	40.0	40.0	0.159
Absolute diff	-20		20		0.002	0		0		0.082

Table 19. Key secondary outcome measures split by baseline LVEF

Abbreviations: LVEF= Left ventricular ejection fraction; SBP= Systolic blood pressure; DBP= Diastolic blood pressure; eGFR= estimated glomerular filtration rate; Diff=Difference; DAPA=Dapagliflozin; IQR=Interquartile range

*= Test of significance used was Wilcoxon signed rank test



LVEDV=left ventricular end diastolic volume; LVESV=left ventricular end systolic volume; LVEDV-i=indexed left ventricular end diastolic volume; LVMI=Indexed left ventricular mass; LVESV-i=indexed left ventricular end systolic volume; LVEF=left ventricular ejection fraction; Hb=haemoglobin; DBP=diastolic blood pressure; BHB=beta-hydroxybutyrate

4. DISCUSSION

4.1 OVERVIEW

The REFORM trial showed that 1 year of dapagliflozin therapy in individuals with T2DM and HF did not result in a significant effect on measures of LV remodelling. Nevertheless, it also showed that the effects of SGLT2-inhibition such as weight loss, blood pressure reduction, increased diuresis, ketogenesis and increased haemoglobin / haematocrit which were previously demonstrated in the T2DM population is maintained in the HF cohort.

Although we were unable to identify any direct ‘morphological’ changes in the heart by way of remodelling, there may be molecular changes such as improved myocardial energetics that could be responsible for the impressive morbidity and mortality outcomes seen in the large CV outcome trials of SGLT2-inhibitors so far. Although this was not directly studied by this work, we did observe surrogates that suggest, at the very least, dapagliflozin therapy provides a suitable milieu to enhance myocardial energy efficiency in the setting of HF. This will need to be confirmed by other dedicated mechanistic trials in the future.

In exploratory analysis, we found a signal suggesting that dapagliflozin may result in LV reverse remodelling in patients with LVEF $\geq 45\%$. There may be two potential mechanisms for this; firstly, we speculate that patients with more advanced LVSD may not have been able to respond to improvements in myocardial energy efficiency conferred by dapagliflozin therapy due to more extensive cardiomyocyte damage. Secondly, our observations may have been

confounded by a disproportionately larger reduction in loop diuretic dose in the LVEF < 45% subgroup on dapagliflozin, which resulted in higher LV loading conditions thereby preventing reverse remodelling.

There were also no serious safety concerns around the use of dapagliflozin in our cohort, with a transient and reversible decline in renal function (which is an expected effect of a diuresis-inducing drug) being the only concern.

4.2 LEFT VENTRICULAR REMODELLING

Changes in parameters of LV remodelling (i.e. LV volumes, EF and mass) have long been shown to be markers of survival in patients with CV disease.(193-195) LVESV in particular, has been shown to be an important predictor of survival in a range of patients with cardiovascular disease, and reducing it is associated with improved outcomes in numerous therapeutic trials.(194,196,197)

In the EMPA-REG OUTCOME trial there was an early divergence of the survival curves for mortality and MACE (at around 3 months), and an even earlier divergence in HF hospitalisation. A similar pattern was seen in the CANVAS trial (for MACE and HF hospitalisation) and DECLARE-TIMI 58 (for HF hospitalisation). This early divergence suggests that SGLT2-inhibition probably has a benefit profile that differs from the classical CV risk reduction by way of improved glycaemia, weight loss and BP control alone. The prime 'mechanistic' candidate was the diuretic effect of SGLT2-inhibitors, which we concluded, should result in reduced LV volumes in the first instance.

This work showed that 1 year of dapagliflozin therapy had no effect on measures of LV remodelling. It is perhaps not altogether surprising as our findings are actually consistent with a number of pre-clinical and clinical studies. Rodent T2DM models using empagliflozin showed improvements in diastolic function but no effect on LV volumes or LVEF. (198,199) In a small, open labelled, uncontrolled clinical trial of 10 patients with T2DM and CV disease (but no HF), Verma et al. demonstrated that 3 months of empagliflozin

therapy lead to improvements in LVMI and measures of diastolic function but not LV volumes or LVEF. This study was limited by the use of echocardiography and a relatively short observation period.(200) In a more recent study by the same group (EMPA-HEART Trial), this time using CMR in a larger cohort of 96 individuals with T2DM and CV disease (6% with HF), they found that 6 months of empagliflozin therapy vs placebo resulted in reduction in LVMI by -2.6 vs -0.01 g/m² (with the greatest improvements occurring in those with higher baseline LVMI). Once again, there was no effect on LV volumes or EF.(201)

The reason why dapagliflozin therapy was neutral on LV remodelling may be twofold: Firstly, the effects of current-generation HF therapy (such as BB, ACE-I / ARB and MRA) on LV remodelling are well documented.(192,202,203) The majority of patients in the REFORM trial were on BB and ACE-I/ARB (>80%) and 41.1% were on an MRA. Although we were unable to determine the duration of these medications, with an average duration of confirmed HF of 6.2 years it would be fair to say that the patients would have been on them for some time. These drug classes have a much more potent effect on ventricular loading than the modest effects seen by SGLT2-inhibitors, therefore any further improvement in parameters of LV remodelling would be difficult to achieve. Additionally, the REFORM cohort only had mildly increased LV volumes, mild impairment of LVEF, and normal LVMI.

Secondly, and perhaps more interestingly, the reason why there was such a striking improvement in HF outcomes in the large trials in spite of a lack of effect on LV remodelling could be because SGLT2-inhibitors act in a novel way

that is not reflected by changes in LV remodelling as we currently understand them. There is evidence demonstrating SGLT2-inhibitors improve myocardial energetics, ion exchange, necrosis and fibrosis pathways which may potentially contribute to overall outcomes.(152,153) We did not study these effects directly but there is indirect evidence from our findings to suggest that dapagliflozin induces changes that may support increased myocardial work efficiency, and this is discussed in the subsequent sections. Animal models show SGLT2-inhibition by empagliflozin affects myocardial sodium / hydrogen exchanger resulting in reduced cytoplasmic sodium and calcium concentration, while increasing mitochondrial calcium.(204) Other rodent and preliminary work on human cardiac fibroblast show dapagliflozin and empagliflozin prevent myocardial fibrosis by suppressing collagen synthesis via various mechanisms such as activation of M2 macrophages, inhibition of myofibroblast differentiation and attenuation of transforming growth factor beta-1 activity.(205,206)

4.3 METABOLIC AND HAEMODYNAMIC EFFECTS

4.3.1 Weight

One of the key benefits of SGLT2-inhibitor therapy in the T2DM population is weight loss. As discussed in section 1.6.2 of the introduction, the proposed mechanism of this weight loss is from calorific loss via glycosuria, increased lipolysis due to a shift in metabolism towards ketogenesis, and to a smaller extent, water loss from increased diuresis.(143,147)

In the REFORM cohort, there was no statistically significant reduction in weight. We saw a nearly 2kg reduction in weight that was approaching significance in the group treated with dapagliflozin when 2 large outliers were excluded from analysis (see Section 2.11.2). When they were included in the regression model, the weight trend remained but statistical significance was lost completely. Interestingly, there was no significant difference in the proportion of fat, as determined by bioelectrical impedance, between the two groups. However, there was an increase in BHB which is a product of fat metabolism and can be considered as a surrogate for increased lipolysis. We believe the reason for this discordance may be due to a limitation in the test itself; bioelectrical impedance estimates the proportion of fat by subtracting fat-free (lean) tissue from total body weight. Indeed a study by Browning et al. on a similar machine (from the same manufacturer as the one used in REFORM) showed that the fat measurement by the machine correlated well to total abdominal fat and waist circumference but not to visceral fat.(207) Visceral fat has been implicated in a number of diseases including IHD, hypertension,

T2DM and insulin resistance, colon, breast and prostate cancer and increased in-hospital mortality.(141)

As SGLT2-inhibitors have been shown to increase lipolysis and have significant improvements in CV mortality, it follows that visceral fat is the likely target of this drug. Indeed a MRI sub-study of a 2-year trial of individuals with T2DM using dapagliflozin suggested a signal of proportionally more visceral fat loss than subcutaneous fat.(140) The reason this was not observed in REFORM could be because visceral fat loss was not detected by the test we performed, and / or the readings were confounded by changes in the non-fat content such as interstitial and intravascular fluid volume as a result of diuresis. Ideally, we should have measured the visceral fat content using MRI which is the current gold standard (but that would have increased the scan duration and the overall cost of the trial).

4.3.2 Blood pressure and heart rate

Patients in the dapagliflozin group had significantly lower diastolic BP and trends towards lower systolic BP and MAP, while there was no difference in their heart rate at the end of 1 year.

It is important to note that the difference in diastolic BP between groups was due to higher pressures in the placebo group while those in the dapagliflozin group had not changed. SGLT2-inhibitors have been shown to improve arterial stiffness and endothelial function, though the mechanisms are currently unclear.(147,152) Additionally, as discussed in section 1.6.3 of the introduction, SGLT2-inhibitors increase sodium delivery to the macula densa thereby indirectly lowering RAAS and sympathetic output. These effects account for the reduction in both systolic and diastolic BP seen in patients with T2DM. However, in the context of the REFORM cohort, these changes on systolic BP may be limited by the other, more potent, anti-hypertensive therapy the patients are already on.

The lack of reflex tachycardia and sizeable reduction in MAP that approached significance is consistent with other work suggesting that SGLT2-inhibitors lower sympathetic outflow and vascular resistance, thereby potentially accounting for the larger effect on diastolic BP seen in REFORM.(208) Nevertheless, it is difficult to draw definitive conclusions on such mechanisms from this trial which was not designed (or sufficiently powered) to evaluate these effects.

4.3.3 Diuresis

We confirmed that dapagliflozin maintained its diuretic effect in the HF population who already were on chronic loop diuretic therapy. This is an important finding because there were concerns around over-diuresis and renal dysfunction when prescribing SGLT2-inhibitors to patients already on loop diuretics. Indeed, when this trial was conceived in 2014 there was no data on the interaction between these drug classes, forcing us to be cautious and designing a protocol that allowed for down-titration of loop diuretics if required.

The diuretic effect of dapagliflozin was reflected by the 28.0 mg/day adjusted mean difference of loop diuretic dose between the two groups. This is a sizeable reduction in loop diuretic requirement considering this is a population of HF patients with a baseline mean loop diuretic dose of 49.8 mg/day that had been stable for at least three months (usually much longer). We also saw half the patients in the dapagliflozin arm either lower or come off loop diuretics completely. This loop diuretic-sparing effect reduces the overall pill burden, which in patients with HF, would be a welcomed change. Additionally, by using a different mechanism to achieve diuresis, it is feasible that SGLT2-inhibitors may be helpful in loop diuretic-resistant patients as well.

Unfortunately, the safety provision in the trial protocol allowing for dose titration of loop diuretic therapy may have also resulted in confounding; there was no significant difference in the total water content, as measured by bioelectrical impedance, in spite of the large reduction in loop diuretic dose. If patients in the dapagliflozin group were left on their usual loop diuretic doses, this may have resulted in lower total water content and even perhaps lower LV volumes

at the end of 1 year. It is also important to recognise that none of the patients on the REFORM trial were on sacubitril/valsartan, which also has diuretic properties. The effects of concomitant SGLT2-inhibitor and sacubitril/valsartan use has not been studied therefore patients on this combination will have to be monitored closely for dehydration and/or worsening renal function.

4.4 MYOCARDIAL ENERGETICS

Myocardial energetics is a new and exciting area of intense interest particularly with regard T2DM and HF. As alluded to in Section 1.3.2 (see Table 5) there is a shift toward the use of FFA as myocardial fuel in T2DM. This is a less efficient fuel substrate and the problem is compounded further in patients with dysfunctional hearts such as HF. SGLT2-inhibitors increase haemoglobin and ketone body levels which could help improve myocardial oxygen delivery as well as offer a more oxygen-efficient fuel substrate, thereby improving overall myocardial work efficiency.(62)(Figure 17)

Indeed, in REFORM we observed a 1.16 g/dL increase in haemoglobin level and 0.04 mmol/L increase of the ketone body BHB, representing a 23.5% increase from the mean baseline value. We are unable to determine the true mechanism of these effects within the scope of this study, however we can infer that increased diuresis contributed to the rise in haemoglobin but, as discussed in Chapter 1.6.6, increased EPO synthesis is another likely factor. Similarly, increased BHB concentration is likely due to the increased lipolysis induced by SGLT2-inhibition, but we are unable to confirm this in the present study.

Although we did not study the effect of these changes on myocardial energetics directly, there is previous work demonstrating that even though skeletal muscle utilisation of ketone bodies is diminished in advanced HF, myocardial ketone body extraction remained unchanged.(209) However, the only way this hypothesis can be tested would be to conduct PET and CMR

studies to directly measure substrate uptake in various conditions using different radio-labelled fuels.

If proven, this could explain why no changes to LV remodelling were seen in REFORM. The effects of SGLT2-inhibition on the major determinants of LV remodelling – ventricular preload and afterload (intravascular volume and blood pressure) – are modest at best. Perhaps all the beneficial effects of SGLT2-inhibition on the myocardium are occurring at the molecular level which are not manifested by morphological changes as measured by features of LV remodelling as we currently understand. Further study into this is warranted.

4.5 EXPLORATORY ANALYSIS

Although there was no effect seen on measures of LV remodelling when the entire cohort was analysed, post-hoc exploratory analysis suggests dapagliflozin reduced LV volumes and indexed mass in patients with LVEF $\geq 45\%$. Importantly, there was no interaction seen in all other key secondary outcomes such as weight, BP, Hb and BHB.

These findings suggest that the cardiac effects of dapagliflozin are influenced by the individual's LVEF when starting therapy; i.e. those with more severe LVSD (as suggested by a lower LVEF) have a poorer response to dapagliflozin with regard to LV remodelling. As alluded to in section 4.2, the ventricular loading effects of dapagliflozin are modest and is likely insufficient, in the case of advanced LVSD, to result in any appreciable difference in LV remodelling. Instead, in the subgroup of patients with LVEF $\geq 45\%$ - which could perhaps be considered as having HFpEF - improvements in LV remodelling were seen because the pathophysiology of HF differs significantly from those with HFrEF.

The so-called ventriculo-vascular decoupling of HFpEF is a key consideration; current understanding suggest there are distinct haemodynamic (ventricular stiffness and fibrosis, left atrial hypertension, pulmonary vascular disease and volume expansion) and molecular (dysfunctional myocardial energetics, microvascular inflammation and cellular/extracellular structural abnormalities) changes involved in the pathophysiology of HFpEF.(210) Perhaps the effects of SGLT2-inhibition on diuresis, myocardial energetics (and other postulated effects on myocardial ion exchange and fibrosis) are more suited to HFpEF

compared to traditional HF therapies such as beta-blockers and RAAS-inhibitors which are more effective in offloading more dysfunctional ventricles.

Indeed this differential effect, preferring HFpEF, may also explain why such striking reductions in HF-related outcomes were seen in the large CV outcome trials which seemingly had very few patients with HF. It is conceivable that many of these patients with T2DM and CV disease (or high CV risk) actually had undiagnosed HFpEF which responded favourably to SGLT2-inhibition.

It is also important to recognise that there was no interaction seen in other secondary outcomes. This means the 'non-cardiac' effects of dapagliflozin (e.g weight loss, diuresis, BP, Hb, BHB) were unaffected by baseline LVEF. In other words, all patients benefitted from these 'non-cardiac' effects, but those with LVEF $\geq 45\%$ had additional benefit by way of improved LV remodelling.

At first glance our findings appear to be in contradiction to the subgroup analysis from DECLARE TIMI-58(166) showing a mortality benefit in patients with LVEF $< 45\%$. It is important to note that unlike REFORM where the LVEF data were all derived from CMR performed at recruitment into the trial, the LVEF data from DECLARE were from differing modalities (echocardiography, CMR, scintigraphy) which were done at varying (unspecified) time-points prior to recruitment. 88% of the patients in the HFrEF group in DECLARE TIMI-58 were on ACE-i/ARB and beta-blockers so it is conceivable that the LVEF at the time of recruitment may well have been different from when the initial test was performed. It is also difficult to draw parallels between these studies because the end-points in DECLARE TIMI-58 was that of CV / all-cause

mortality after a four-year observation, while REFORM studied measures of LV remodelling after 1 year. The mortality reduction in the predominantly NYHA functional class I and II patients of DECLARE would have likely been driven by reduced sudden cardiac death from arrhythmias (rather than pump failure). This could be attributed to the improved intracytoplasmic ionic milieu and reduced fibrosis from SGLT2-inhibition, as discussed at the end of chapter 4.2.

Another important consideration that was uncovered by the exploratory analysis was that of the potential confounding effect of loop diuretic dose changes during the trial. We first confirmed that SBP reduction was associated with reduced LV volumes and mass for the entire cohort. We then identified that there was greater BP reduction in the higher LVEF subgroup compared to those with lower baseline LVEF. Importantly, there was no interaction between treatment allocation and LVEF grouping, suggesting that the effect of dapagliflozin was not modified by baseline LVEF. Further analysis revealed the reason behind this difference in BP effect between subgroups was due to a greater reduction in loop diuretic dose in the lower LVEF group. By play of chance, patients on the dapagliflozin arm of the lower LVEF subgroup had the highest loop diuretic dose and lowest eGFR at recruitment. (Section 3.10, Table 19) The additional diuretic effect of dapagliflozin resulted in an expected decline in renal function, triggering the safety protocol to scale back on loop diuretic dose. This could potentially explain the lack of BP reduction, and in turn, the neutral effect on LV remodelling in this subgroup.

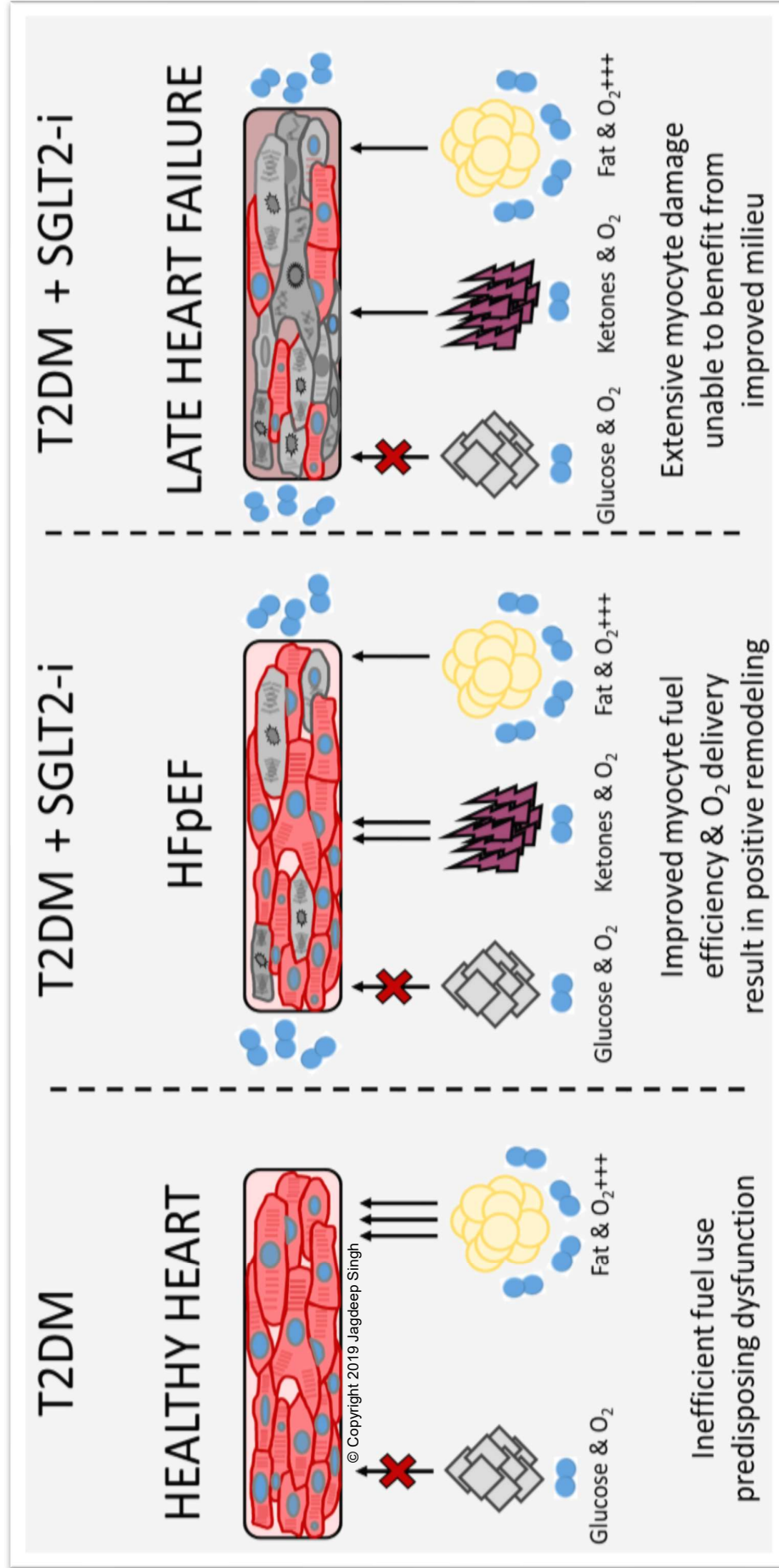


Figure 17. Proposed mechanism of differential effect of SGLT2-inhibition.

SGLT2-i=Sodium glucose-linked cotransporter 2 inhibitor; HFpEF=Heart failure with preserved ejection fraction; O₂=Oxygen; +++=Large amount

4.6 EXERCISE TESTS

There was no difference in all major measures of CPET and the 6MWT distance between groups in REFORM. Although the mean baseline peak VO_2/kg measurement may appear low at first glance, it is important to note that the mean respiratory exchange ratio was less than 1.0 indicating that it was a submaximal effort test.

Improvement in peak VO_2/kg has been associated with higher survival, but this is in the context of transplant candidates.(211) The prognostic value of peak VO_2/kg starts to diminish the less severe the degree of HF. In an interesting trial-level meta-analysis of more than 70,000 individuals by Wessler and colleagues showed that drug or device-induced improvements in peak VO_2 and 6MWT did not correlate to the long term mortality outcomes of those interventions in patients with HFrEF.(212)

Of course, the goal in REFORM was to determine if there was any objective improvement in measures of exercise tolerance; and we found none. Unfortunately, there were procedural shortcomings in conducting the final CPET for 6 patients (representing 12.2% of the per-protocol population) requiring exclusion from analysis which may have diluted the overall effect in an already underpowered cohort.

4.7 QUALITY OF LIFE

A SF-36 score of 50 for PCS and MCS is considered average, while a MLHF score of <24 is considered good. The REFORM cohort started with scores that reflected a moderate quality of life. After 1 year of dapagliflozin therapy there was no difference in the general (SF-36) and disease specific (MLHF) quality of life measures.

It is perhaps not unexpected that there wasn't any change in the perceived quality of life in this cohort as the changes seen in other clinical parameters (eg weight, BP etc) were modest. It is likely that there will have to be a sustained effect for a longer duration before patients will start to notice a perceptible change in their physical or mental wellbeing.

4.8 BLOOD AND URINE BIOCHEMISTRY

4.8.1 Amino-Terminal Pro B-Type Natriuretic Peptide

NT-proBNP was unexpectedly higher in the placebo group at baseline. Importantly there were no differences in the baseline CMR-derived LV volumes or EF, NYHA functional class, BP or renal function between the groups. Large inter and intra-individual variation in NT-proBNP concentrations have been confirmed in patients with chronic HF and can sometimes complicate analysis.(213,214) Indeed some authors have made the observation that, paradoxically, trials with the most benefit in NT-proBNP-guided therapy have had the least stringent NT-proBNP targets.(215) This is reflected by the ongoing uncertainty on the best cut-off values of BNP / NT-proBNP particularly in chronic HF.

The final NT-proBNP concentrations were not significantly different but remained heavily skewed. This makes interpretation very difficult; was there an improvement in the placebo group which started out with higher concentrations of NT-proBNP but then ending up no different from the dapagliflozin group? This, of course, goes against data suggesting SGLT2-inhibitors are beneficial in HF.(167) Furthermore, there is no evidence within this study itself that suggest patients in the placebo group did better in any of the other parameters such as CMR-derived LV volumes or EF, NYHA functional class, BP (which improved in the dapagliflozin group) or renal function.

Perhaps having a NT-proBNP cut-off in the inclusion / exclusion criteria may have mitigated this problem but it would have made recruitment much more difficult. This is discussed further in the study limitations.

4.8.2 Oxidized LDL

There have been observations of increased LDL and HDL (with preserved ratios) in individuals treated with SGLT2-inhibitors.(163) As discussed in section 1.6.5 above, SGLT2-inhibitor shifts metabolism towards increased lipolysis. The activation of lipoprotein lipase and hepatic triglyceride lipase results in increased small dense LDL which are more prone to oxidation than the large buoyant type of LDL.(216) This could explain our observation of higher oxi-LDL levels in the dapagliflozin group.

Of course, oxi-LDL has been implicated in the pathophysiology of a number of chronic diseases including metabolic syndrome and atherosclerosis. In those scenarios the metabolic pathways involved in the generation of oxi-LDL are quite different to those generated by SGLT2-inhibitor.(217) It is important to recognise that oxi-LDL are merely surrogate markers of the various biochemical processes occurring in the body. In the case of this study it may just be an indicator of increased lipolysis induced by SGLT2-inhibition.

4.8.3 Haemoglobin A1C

There was no significant difference in the HbA1c levels between both groups. The efficacy of dapagliflozin in lowering HbA1c has been confirmed; 10mg once daily dapagliflozin alone or in combination with other anti-diabetes medications reduce HbA1c between 0.58% to 1.11% in patients with T2DM.(133) Although HbA1c was a secondary outcome measure, the REFORM trial was not sufficiently powered to assess this.

Interestingly, subgroup analysis from the EMPA-REG trial showed that the efficacy of empagliflozin in reducing CV mortality, all-cause mortality and HF hospitalisation was independent of the degree of change in HbA1c. Indeed, it was seen even when HbA1c reduction did not meet thresholds for clinically meaningful reduction as defined by the ADA, EASD or US-FDA.(218)

This suggests that the cardioprotective effects of SGLT2-inhibitors are unrelated to glycaemic control and perhaps in the future, SGLT2-inhibitors may no longer be classed as a diabetic drug.

This revisits the perennial debate regarding the role of glycaemic control in CV outcomes. Indeed the VADT,(80) ADVANCE(81) and ACCORD(82) trials are some that have shown no benefits, and in some cases increased harm, amongst those undergoing intensive glucose lowering therapy. The mechanisms behind these findings are outside the scope of this work.

4.8.4 Urinary sodium

Urinary sodium excretion was significantly lower in the dapagliflozin group. Although one would expect an increase in urinary sodium excretion in this group due to the mechanism of action of dapagliflozin (as discussed in section 1.6.3), this was not observed.

Although appearing paradoxical at first glance, the simple explanation for this observation is the change in furosemide dose. As noted in section 3.6, there was a near halving of the average daily loop diuretic dose and 50% of patients in the dapagliflozin arm had either reduced or stopped loop diuretic therapy. Loop diuretics have a potent natriuretic effect and this is the very likely cause for this 'unexpected' observation.

4.9 SAFETY

4.9.1 Renal function

There were more cases of major worsening of renal function in the dapagliflozin group (8 vs 0; $p=0.008$). Major worsening was arbitrarily defined as more than 20% increase in creatinine or eGFR less than 45 ml/min/1.73 m² in two readings at least 1 week apart. It is important to note that this derangement in renal function was reversible by reducing the loop diuretic dose and resolved in all 8 cases without the need to withhold trial medications.

Indeed, all SGLT2-inhibitors cause an initial rapid decline in eGFR which then recovers gradually.(219) The mechanism for this is the increased tubuloglomerular feedback due to higher sodium delivery to the macula densa (as a result of inhibition of the SGLT2 transporter in the proximal convoluted tubules). This feedback results in selective vasoconstriction of the afferent glomerular arterioles which reduces renal blood flow, thereby lowering the transglomerular pressure gradient. The lower pressure results in a lower eGFR but this does not necessarily imply renal dysfunction, in fact this is a reno-protective effect.(220-222) ACE-I / ARB achieve reno-protection via a similar mechanism of lowered transglomerular pressure by selectively dilating the efferent arteriole. Indeed, when both drugs are used together, they could cause a significant drop in the transglomerular pressure, but this plateaus and recovers over time.

As this was the first time SGLT2-inhibitor therapy was used in the HF population, in which renal dysfunction is fairly common, (and also because the

lower limit for use of dapagliflozin is 45ml/min/1.73 m²) we wanted to ensure safety was prioritised, hence why we had to act when a participant fulfilled criteria for major worsening renal function.

4.9.2 Genital infections / urinary tract infections

Genital infections are an important concern when using SGLT2-inhibitors and can, in some instances, result in significant morbidity and even mortality. The US-FDA recently issued a warning for increased risk of Fournier's gangrene - an extremely rare form of necrotising fasciitis, usually in males, affecting the perineum that frequently requires wide surgical debridement and is sometimes life threatening. They report 12 cases of Fournier's gangrene in patients taking SGLT2-inhibitors the 5 years between 2013 and 2018. Five patients were female, and all required surgical debridement (some requiring multiple disfiguring surgeries) and one patient died. In contrast, there were only 6 cases of Fournier's gangrene (all male) in the last 30 years in all other classes of anti-diabetic therapies combined.(223) Gadzhanova and colleagues also demonstrated the risk of genital infection such as vulvovaginal infections, vulval abscesses, balanitis, male and female candidiasis was 3.5 times more likely with the use of SGLT2-inhibitors compared to DPPIV-inhibitors, while there was no difference in the incidence of UTI.(224)

In REFORM, the small cohort and short observation period meant that there was no statistically significant difference in the incidence of genital infections or UTI, although there were numerically more instances of these (5 vs 2) in the dapagliflozin arm. Two patients had recurrent episodes of infection, both were in the dapagliflozin arm. All instances were treated by GPs using standard anti-bacterial / anti-fungal therapy over the usual treatment duration. Study drug was not withheld.

4.9.3 Others

There was no difference in the incidence of all-cause deaths, CV deaths or acute coronary syndrome between groups, if anything there were more instances of these events in the placebo group.

There was one instance of cancer recurrence in the dapagliflozin group and one case of new diagnosis of cancer in the placebo arm, none were breast or bladder cancers which were an initial concern in the preclinical stages of dapagliflozin and canagliflozin development.(225)

We did not observe any instances of euglycaemic diabetic ketoacidosis or fractures which are ongoing safety concerns in the use of SGLT2-inhibitors.

5. STUDY STRENGTHS AND LIMITATIONS

5.1 STUDY STRENGTHS

5.1.1 Trial design

The REFORM trial was designed specifically to test the mechanistic effects of dapagliflozin in a population of patients with HF. The design was robust and comprehensive as it looked at various aspects of HF which included the current gold-standard of CMR-derived measures of LV remodelling, a battery of biomarkers, body composition analysis, measures of effort tolerance as well as quality of life measures. The observation period of 1 year is more than other similar studies of LV remodelling which range between 3 to 6 months,(200,201) allowing for adequate time for remodelling to fully manifest itself, and more importantly, that it is sustained after 1 year of therapy.

5.1.2 Reliability and reproducibility of cardiac MRI

CMR is the current gold standard for measuring parameters of LV remodelling. It has two distinct advantages over echocardiography; firstly, its protocol-based image acquisition ensures high fidelity measurements between the two readings a year apart as well as reduces the intra-observer variability. Secondly, it generates highly accurate and reproducible measurements that reduces the inter-observer variation and allows for a smaller sample size while maintaining statistical power.

5.1.3 Breadth of biomarkers measured

The REFORM trial measured a host of blood and urine markers, allowing for a comprehensive analysis of the biochemical changes induced by dapagliflozin in the context of HF. It enhanced the safety of the trial (with regular NT-proBNP measurements in all visits allowing for early detection of HF decompensation), allowed for a possible explanation for some of the unanticipated observations (e.g. differential effects of dapagliflozin due to improved myocardial energetics from increased ketosis) as well as generating some new avenues of research (e.g. urinary sodium excretion and the effects of renal free water and solute handling with concomitant use of loop diuretics and SGLT2-inhibitors, which is a study currently being conducted by our research group).

5.2 STUDY LIMITATIONS

5.2.1 Adjustment of loop diuretic dose

One significant limitation of the REFORM trial was to do with the adjustment of loop diuretic dose. As this was the first clinical trial conducted in a cohort of patients with HF who were on established loop diuretic therapy, we were required by the MHRA to be cautious and include the allowance to modify (up or down titrate) the concomitant diuretic therapy. (At the time of conception of this trial, manufacturers recommended avoiding concomitant use of loop diuretics and SGLT2-inhibitors due to lack of data on the diuretic effects of SGLT2-inhibitors). Indeed we did see half of patients in the dapagliflozin group needing to reduce or stop their loop diuretic therapy, primarily due to transient impairment of renal function (which we now know, is due to increased tubuloglomerular feedback and is simply part of the pharmacodynamics of SGLT2-inhibition – see discussion on renal safety in section 4.9.1).

Unfortunately, this may have also confounded the overall volume and blood pressure effects of dapagliflozin and could be the reason why no difference was seen in the LV volumes, mass and total body water composition between groups. (see discussion on diuresis in section 4.3.3)

5.2.2 Heterogeneity of heart failure severity

The eligibility criteria for the REFORM trial was a prior diagnosis of HF with echocardiographic evidence of at least mild LV dysfunction (LVEF<45%). The trial protocol did not require a screening echocardiogram to be performed prior to recruitment and, because the CMR images were only batch-analysed at the end of the trial, patients who strictly did not fulfil criteria *at the time of recruitment* could not be identified. With an average duration of HF of 6.24 years, and a majority of the cohort on evidence-based anti-HF therapy, it is conceivable that some patients may have had improvements in their LV function since their last clinic echocardiogram. This was reflected by the wide range of CMR-derived LVEF at baseline (20.0% to 75.1%). A further reason for this discrepancy may be the result of operator variability and lower accuracy of echocardiography compared to CMR.

This heterogeneity in the severity of LV dysfunction may have diluted the power of the primary outcome measure, contributing to the overall neutral outcome. Perhaps mandating a screening echocardiogram or including a baseline CMR exclusion criteria may have mitigated this problem. However, if the findings of the exploratory analysis hold true, (i.e. there is a differential effect of dapagliflozin favouring HFpEF) then having a cohort of homogenously poor LV function would not have identified this effect – this ‘shortcoming’ in recruitment may well have been a blessing in disguise.

6. FUTURE DIRECTIONS AND CONCLUSIONS

Given the unexpected but consistent benefit of SGLT2-inhibitors in the HF population, it is tantalising to consider if these benefits persist in the non-diabetic HF population. Indeed, the DAPA-HF (NCT03036124) and EMPEROR-Reduced (NCT03057977) trials are large multicentre studies looking at the effects of dapagliflozin and empagliflozin respectively on hard outcomes such as CV death and HF hospitalisation in an exclusively HFrEF population regardless of whether T2DM is present or not.

There is also interest in the notoriously difficult to treat population of HFpEF; with the PRESERVED-HF study (NCT03030235) using dapagliflozin in patients with HF and LVEF>45% plus elevated natriuretic peptides (regardless of diabetes status) to look for changes in NT-proBNP concentration and echocardiographic parameters over a 12-week period. The far more ambitious EMPEROR-Preserved (NCT03057951) trial is looking into a similar population, using empagliflozin, with a composite primary outcome of CV death or HF hospitalisation over a 38-month period. If such a benefit is seen, this will be the first drug to have a benefit in hard outcomes in the HFpEF population. Perhaps the signal of potential benefit in this population that was identified in the REFORM cohort will encourage even more interest in this area.

There are also some mechanistic studies looking into the cardio-renal effects of SGLT2-inhibition. The EMPA study (NCT03027960) is performing a detailed analysis of natriuresis (using ion selective electrodes) and blood volumes

(using radio-labelled albumin) in a small group of patients with HF and stable loop diuretic therapy. The RECEDE-CHF (NCT03226457) trial is another project by our laboratory studying the combined effects of empagliflozin and loop diuretic therapy on the renal free water and sodium handling characteristics of patients with HF.

In conclusion, this work has explored the use of SGLT2-inhibitors in patients with T2DM and HF. By conducting the REFORM trial we confirmed, for the first time, that the effects of dapagliflozin on weight, BP, diuresis, Hb/Hct and ketones that were previously demonstrated in the T2DM population has remained consistent in patients with HF as well. However, there was no effect on the measures of LV remodelling, although there was a signal for potential benefit in a subgroup of patients with LVEF $\geq 45\%$. Some of the hypothesis-generating findings of REFORM are already being investigated, such as the cardio-renal effects of uninterrupted loop diuretic therapy with SGLT2-inhibitor as well as the potential benefits of SGLT2-inhibition in patients with HFpEF. The REFORM trial was the first to ask the question about potentially using the SGLT2-inhibitor class in HF and has paved the way for future study into this and, perhaps, even the future 're-designation' of this drug class into a primarily HF indication.

7. PRIZES, PUBLICATIONS AND PRESENTATIONS

Prizes:

Winner of **Fitzgerald Peel Prize** with an award of £500 for best abstract at conference, Scottish Society of Physicians Autumn Meeting 2018.

Publications:

(a) Newsprint:

Buchan L. 'Scots scientists find sweet spot for diabetes treatment', **The Scotsman**. 14 December 2015 (<http://www.scotsman.com/news>)

(b) Scientific publication:

JSS. Singh, A. Fathi, I. Mordi, M. Mohan, G.J. Houston, E.R Pearson, A.D. Struthers, C.C. Lang. Research into the Effect Of SGLT2 inhibition on left ventricular Remodelling in patients with heart failure and diabetes Mellitus (REFORM) Trial Rationale and Design. **Cardiovascular Diabetology**, 2016; 15:97. DOI 10.1186/s12933-016-0419-0

Presentations:

1. **American Diabetes Association** 78th Scientific Sessions (2018) – Oral presentation. (Diabetes Jul 2018, 67 (Supplement 1) 256-OR; DOI: 10.2337/db18-256-OR)

2. **European Society of Cardiology Congress** (2018) – Poster presentation European Heart Journal. 2018; 39 (suppl1) (doi: 0.1093/eurheartj/ehy564.P905)

3. **Scottish Society of Physicians** Autumn Meeting 2018 – Oral presentation.

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9. APPENDICES

9.1 PATIENT INFORMATION LEAFLET



PARTICIPANT INFORMATION SHEET

Title of Study

The REFORM Trial - Research into the Effect Of SGLT2 inhibition on left ventricular Remodelling in patients with heart failure and diabetes Mellitus

Name of Researcher

Chief Investigator: Professor Chim Lang

Principal Investigator: Dr Jagdeep Singh

Details of Study

You are being invited to participate in a clinical trial being sponsored by the University of Dundee and NHS Tayside. This study will form part of a higher doctorate in medicine degree for Dr Singh. Before you decide whether or not to take part it is important for you to understand why the research is being carried out and what it involved. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is unclear or if you would like more information. Take your time to decide whether or not you would like to take part.

Background

People with diabetes are at increased risk of developing heart failure (HF) which can lead to increased shortness of breath, reduced ability to exercise and in some cases premature death as the heart becomes less efficient at pumping blood around the body.

Drug options to treat heart failure in diabetes are currently limited as many of the drugs used are not suitable in diabetic patients as they cause unpleasant or harmful side effects.

A new class of anti-diabetic drugs (SGLT2 inhibitors) may have some benefit in heart failure in diabetes as they may reduce the workload on the heart. It has been shown in previous studies that they may also promote weight loss and increase exercise capacity.

This study will test if there is a real benefit from using a drug called dapagliflozin, (an SGLT2 inhibitor), on improving the efficiency of the heart and improving exercise capacity in patients with diabetes and HF.



The findings of this study may help to find out if this class of drugs, (SGLT2 inhibitors), are useful in diabetic patients with HF.

If you take part you will have a Magnetic Resonance Imaging (MRI) scan of the heart, using an MRI machine, to measure the size and extent of thickening of the heart muscle before starting on treatment of dapagliflozin, a SGLT2 inhibitor, or placebo for one year. You will also do exercise testing on an exercise bike (if capable) and a walking test plus fill in some questionnaires on how heart failure affects your quality of life.

As this is a clinical trial all participants will be randomly allocated to either dapagliflozin or a dummy medication (placebo) so that the researchers can compare if there is a difference between normal treatment and addition of dapagliflozin. You will continue to take all currently prescribed medication for your diabetes and heart failure. However, if you are on insulin, we will reduce your total insulin dose by 10% on the day we start you on the trial medication (Visit 2). You will have a further MRI scan and the other tests mentioned above when your one year treatment with dapagliflozin or placebo finishes.

Do I have to take part?

Participation in this study is entirely voluntary and you are free to refuse to take part or withdraw from the study at any time (without having to give a reason) and without this in any way affecting your future medical care or your relationship with medical staff looking after you. Some insurance companies consider that participation in medical research such as this is a "material fact" which should be mentioned in any proposal for health-related insurance or which could influence their judgement in consideration of claims under existing policies. You should check that participation in this research does not affect any policy you might be thinking about taking out or any existing policy.

What is involved in the study?

This study takes between 12 to 13 months for you to complete with visits for the study scheduled to take place at your convenience. This study is a randomised, double blinded, placebo controlled single centre study to be conducted at Ninewells Hospital & Medical School, Dundee. You will be given a tablet which contains the medication we are testing (called dapagliflozin) or an inactive tablet (called a placebo). Before you start on any study medication the doctor will do a screening visit to check you are eligible for the study. A summary of the tests done is included in a diagram at the end of this information sheet.



Visit 1 Screening

At the screening visit the doctor will assess your suitability for the trial. Typically this may involve a visit of up to one hour. At the screening visit the doctor will check your medical history and do a clinical examination with the purpose being to ensure you are suitable to take part in the study. The doctor or research nurse will check your blood pressure, height, weight and pulse readings and do some routine blood tests to assess your liver, kidney function and check you are not anaemic and to find out how well your diabetes is controlled to be sure that it is safe for you to participate. A blood test for BNP will also be taken which is test that can tell us how severe your heart failure might be.

The total volume of blood taken at this visit will be approx. 22ml (4 teaspoons full).

If it is safe for you to proceed you will be asked to return for an MRI Scan- a special scan of your heart (further details below). If anything is discovered in your screening visit that is of concern and prevents you from taking part we will arrange the appropriate medical follow up for you.

If at Visit 1 you are found to have a history of a penetrative eye injury or exposure to metal fragments in your eye(s), that required medical attention, you will be advised that it is unsafe for you to continue further in this study as there is a risk that the magnetic field in the MRI scan could move the metal fragment which may cause harm to your eye. With your consent, we will write to your GP informing them of your MRI safety status, as this information may be of benefit for your future health care needs.

Baseline MRI Scan

For the MRI Scan you will receive an appointment either by telephone call, letter or e-mail and be sent directions to attend the MRI department of the Clinical Research Centre, Ninewells Hospital, Dundee.

If you are a woman of child bearing potential you will be asked to provide a urine specimen which you can either bring with you (a specimen bottle will be provided) or you could provide a sample at the beginning of your MRI clinic visit. A pregnancy test will be performed to ensure your safety. A positive result will exclude you from having an MRI scan.

Before your scan you will meet one of the research team who will check that you are eligible to have the scan and who will obtain your written consent. You will then be seen by the radiographer, the person taking your scan, and she/he will help you to complete a checklist about matters that might prevent you from having the scan.



If at Visit 1 you are found to have NO history of a penetrative eye injury and have had NO exposure to metal fragments in your eye(s), or otherwise the radiology staff establish that it is SAFE to scan you, will may proceed to have your MRI whereupon you will then be asked to change into a gown for the scan. After being prepared for your scan, you will be asked to lie up on the scanning table and then will be moved into the centre of the scanner (the scanner is shaped like a big doughnut). During the scan, which takes around 45 minutes, you will be able to speak to the radiographer. The scan will take pictures of your heart and blood vessels. As the scan is noisy you will be wearing hearing protection. After you have completed the scan you are free to go home. You can drive if you need to or if you prefer a taxi can be arranged to take you to and from the hospital. A specialist will examine your scan at a later date for any signs of disease and will measure your left ventricular mass.

This MRI visit should take no longer than one and a half hours.

Visit 2 Baseline

This visit will take place after the MRI Scan has been done anytime up to two weeks before or after the MRI scan but usually on the day of it. It will include the following investigations:

Vital signs: checks of your blood pressure, pulse, height and weight.

Blood and urine tests: Research bloods (20ml- 4 teaspoons full) will be taken and stored for analysis after the trial has ended. Total blood taken will be 42ml. at each visit where all bloods; both research and safety plus BNP are taken (approx.8 teaspoons) and 22 ml when safety bloods & BNP only are taken. BNP blood tests tell us how well the heart is responding to treatment for heart failure. Additionally, we will also collect a sample of your urine to test its protein and sodium content. This will be repeated at the end of the study. Following completion of the trial we may test for additional markers of interest on any left-over blood which will be stored anonymously in the secure Dundee University laboratory in the division of Cardiovascular and Diabetes Medicine for up to ten years.

Genetic analysis blood test: You will be asked to provide one 10ml blood sample for storage. The sample will be fully anonymised and will be subject to the approval of a Research Ethics Committee prior to future analysis. The results of any future genetic tests would not be linked to your records and you would not receive any information about the results. You can opt not to have this done without affecting your participation in the study. This sample will be stored in the secure laboratory within the Division of Cardiovascular and Diabetes Medicine at Ninewells Hospital.



Exercise Bike Test: You will be asked to do a test on an exercise bike where we will ask you to cycle and will measure your heart rate and how much oxygen you use up and what gases you breath out when exercising. You will be connected up to a heart monitor and a gas mask to get these measurements done and you should stop when you have tired.

Six minute walk test: You will be asked to do two six minute walk test, at your own pace, along an even floored corridor to see how far you can walk back and forth on a 25 metre circuit in a six minute period. There will be an hours rest between both tests. If you are unable to walk for six minutes you can stop and we will record the time you walked for and how far you managed.

Quality of Life Questionnaires: You will be asked to complete two short questionnaires which tell us how your heart failure affects your quality of life.

Measuring fluid status by bioelectrical impedance analysis: This is a non-invasive method of measuring body fat and how much water is in your body. You will stand on a specially designed weighing scale with small metal plates on them. These plates generate and detect very small amounts of electrical current. The current generated is very small and you will not feel any sensations during this test which only lasts 1-2 minutes. This short test will be repeated during all your visits to allow us to track the fat and fluid changes in your body in a progressive manner.

At the end of this first drug dosing visit you will be randomly assigned to either dapagliflozin (10mg) or placebo so that the tablets allocated to you are decided in a random way (a bit like tossing a coin) such that neither you nor the research staff will know which tablet you are taking at any time until after the study is completed. This ensures that the study results cannot be influenced by knowing whether you are receiving the medication or not.

You will then be given enough study drug to take once daily for two weeks.

Visit 3, 5 & 7

Visit 3 will occur approx. 2 weeks after visit 2, with visits 5 and 7, 2 month and six month from visit 2. At these visit you will have blood tested for safety measures, have your vital signs measured and the doctor will assess if you have had any problems on the study medications. We will also perform the



bioelectrical impedance test at each visit. At the end of each visit you will receive a further supply of study medications to last you until the next visit.

Final Visit 9

This is the final study visit. At this visit the final safety and research blood and urine tests will be done, you will have a repeat MRI scan (within 2 weeks of the visit) and do a further six minute walk test, exercise bike test and have your fluid levels measured which all will ultimately be compared with the baseline measurements taken before you started on study medication. With your permission we will also take an extra 20ml sample of blood for research purposes. This sample will be stored in the secure laboratory within the Division of Cardiovascular and Diabetes Medicine at Ninewells Hospital.

For all visits noted above the doctor will assess you for any side effects of the medication and will check your vital signs and do blood tests to assess if the dapagliflozin has caused any problems.

Telephone contact: visits 4, 6 and 8

There are three visits scheduled where the doctor or research nurse will phone you at home or on your mobile. These are scheduled to occur after 1 month, 4 months and 9 months from visit 2. The doctor or nurse will check how you are doing on the study medication and if there are any concerns they may bring you up to Ninewells for further investigation as required. You will of course have their details to contact them at any time during the study if you are concerned or unwell on the medication.

Early discontinuation visit

If for any reason we need to stop your study medications or you wish to come off them, it would be very helpful if you could attend for a final 'early discontinuation' visit. At this visit all the tests you agree to undergo that are scheduled for the final visit will be performed. This is important as it gives the investigators information on the safety of the drug and further information on the effects of the drug on these tests. It also allows the investigator an opportunity to ensure any follow up that may be required from your GP or hospital consultant is planned properly and you are aware of what is being done. You do not though have to attend this visit.

Medication being tested

The medication used in this study is called dapagliflozin. It is used for the treatment of type 2 diabetes and has been licenced in Europe for that since 2012. It works by lowering blood glucose (sugar) by



increasing the amount of glucose removed by the kidneys. Among the known side effects of dapagliflozin are diabetic ketoacidosis (DKA) (a build-up of acid in the blood), hypoglycaemia (low blood sugar), urinary tract infection, genital tract infection and increased urine production. The most serious risks are also the rarest; namely DKA (affecting between 1 in 1000 to 1 in 10,000 patients) and major hypoglycaemia (affecting between 1 in 200 to 1 in 250 patients). To mitigate this small risk even further, we will give you information on the symptoms of DKA to look out for, and we will be monitoring for these and other features of DKA during every visit. To address the risk of hypoglycaemia, participants taking insulin will have their insulin doses reduced by 10% when they join the trial. Their laboratory-based blood sugar levels (along with home-monitored levels) will then be monitored regularly by the study team and GP, and the necessary dose titration will be done. We will be providing you with written information on the possible symptoms of hypoglycaemia and how to manage it. Other common non serious side effects are: Runny or stuffy nose; sore throat.

It has a good safety record and is generally well tolerated. However, like most medicines, dapagliflozin occasionally causes side effects.

The complete range of reported side effects is set out in a Package Information Leaflet, a copy of which will be given to you at your screening visit for your information. This will be further discussed with you before you make a final decision about taking part in this study.

Contraceptive Advice

Anyone who is pregnant cannot take part in this study. If you are a woman of childbearing age we will need to do a pregnancy test before the study. It is also important that you do not become pregnant during the study. We will do a urine pregnancy test at all clinic visits if you are a woman of childbearing potential and not practicing one of the types of contraception or abstinence noted below. Here is some advice on contraception. To avoid getting pregnant, not having sex at all is obviously effective. If you follow this strictly, no contraception is needed.

If not, these are effective types of contraception:

- Combined Oral Contraceptive Pill
- EVRA-osetrogen and progestogen: 'Transdermal Patch'
- Progestogen only pill: 'mini pill'
- Depoprovera injection (medroxyprogesterone acetate)
- Implanon Implant (Etonogestrel)



- Mirena Coil (Intra-Uterine System)
- IUD-copper containing intrauterine device
- Female sterilisation

Male vasectomy is also a good form of contraception but only if the procedure has been checked afterwards by your doctor to make sure it has worked.

No contraception method is 100% reliable by itself. Even surgical sterilisation in men and women has been known to fail very occasionally. We advise using additional contraception from the start of the study.

You may normally use 'barrier methods' such as the condom, diaphragm or cap. There is no definite proof that using a spermicide with a 'barrier method' gives extra protection but some condoms are manufactured with spermicide on them. If you require further advice on contraception, please ask.

What are the discomforts, risks and side effects?

The side effects of the dapagliflozin are discussed under the 'medication' section above.

Having blood tests taken can cause some mild bruising. The exercise tests may make you tired or short of breath but can and will be stopped if this becomes too uncomfortable for you.

MRI scanning: This type of scan is very safe and does not use radiation. Some people, when being scanned, may feel a bit closed in but you will be in constant contact with the person performing the scan and you can come out at any time. The scanner is a bit noisy but you will be given ear protection which also plays music.

What are the benefits of taking part in the study?

You will be monitored closely during the study and will be seen by a doctor with a special interest in cardiology at each of your study visits. Besides having tests that have already been mentioned, your medication will be reviewed on a regular basis. The tests will give us information about the function of your heart, kidneys and blood circulation. If any of these investigations, including information from the MRI scan of your heart, reveal any new abnormality we will either discuss this with your hospital consultant or refer you to a specialist clinic (whichever seems most appropriate). The study will not immediately benefit you, but if the results of the study are positive it may change the practice of managing patients with diabetes and heart failure, like you and potentially will have a great impact on other such patients in the future. If so, you may gain eventually from our discovering a new treatment for your condition.



Complaints, Insurance and indemnity

Right to raise concerns

If you have any concerns about your participation in the study you have the right to raise your concern with a researcher involved in conducting the study or a doctor involved in your care.

Right to make a complaint

If you have a complaint about your participation in the study, you should first talk to a researcher involved in the study. However you have the right to raise a formal complaint. You can make a complaint to a senior member of the research team or to the Complaints Officer for NHS Tayside.

Complaints and Claims Manager

Complaints and Advice Team

Level 7, Ninewells Hospital

Dundee DD1 9SY

Freephone: 0800 027 5507

Email: nhstaysidecomplaints@thb.scot.nhs.uk

Right to make a claim

In the event that you think you have suffered harm as a result of your participation in the study there are no automatic financial compensation arrangements. However, you may have the right to make a claim for compensation against the University of Dundee or NHS Tayside. Where you wish to make a claim, you should consider seeking independent legal advice but you may have to pay for your legal costs.

Insurance

The University of Dundee maintains a policy of professional negligence clinical trials insurance which provides both legal liability cover and no fault compensation in respect of accidental injury. Tayside Health Board is a member of the Clinical Negligence and Other Risks Insurance Scheme which provides legal liability cover.



Genetic testing

You should be aware that if you apply for health insurance you may be asked questions about your health, including medical history, pre-existing medical conditions and if you have had any genetic test. If you have a diagnosed medical condition, even where the condition is diagnosed as part of a clinical research study, the insurer may take this in to consideration when deciding whether to offer insurance to you.

Participation in this study **DOES NOT** constitute a “genetic test” as defined by insurance companies. Your data will remain confidential unless we are legally required to disclose information by Court order or by statute.

Will the research influence the treatment I receive?

The research will not immediately alter the regular treatments you currently receive.

Will my taking part in the study be kept confidential?

Your personal data will be kept confidential. With your permission, identifiable information about you and data collected during the study will be held securely by the University of Dundee and under the control of the Chief Investigator. All data collected in this study will be coded and stored on a computer system protected by a password only available to the researchers. No one outside the research team will have access to any identifiable information and all identifiable information and data will be kept securely. Your data will be archived securely for at least five years after the end of study as this is a legal requirement for drug studies. With your permission, we will inform your GP of your participation in this study. It is a requirement of the regulatory authority for clinical trials that your records in this study, together with any other relevant medical records, be made available for scrutiny by appropriate staff from NHS Tayside, University of Dundee (or their appointed third party) and the regulatory authority themselves.

Additionally there will be two sets of information obtained after you have had your MRI scan. One set will be the MRI scan images and the other, the research data obtained from those images. The MRI images obtained will be stored indefinitely using your name and unique hospital record number within the NHS clinical system and can be made available to specialist doctors for your future health care needs. Your research data will be stored using a unique study code which is non-identifiable and held



on password protected University of Dundee secure databases. Only individuals directly involved with the study will have access to this information.

Will I continue to receive the medication used in this study after it finishes?

No. The study is designed to give an indication of possible benefit from the medicine being tested and it may be some time before we can be sure about how useful it actually is.

Expenses

Taxi transport, or reasonable costs to cover your travel costs, will be provided for any extra visits to the hospital for the purposes of this study.

Who has reviewed this study?

The East of Scotland Research Ethics Committee, (REC 2) which has the authority to scrutinise proposals for medical research on humans, has examined this study and has raised no objections from the point of view of medical research.

It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from The University of Dundee, NHS Tayside and by the Regulatory Authorities, whose role it is to check that research is properly conducted and the interests of those taking part are adequately protected.

Contact details for the study Doctor.

If you are worried at any time about the research or wish to discuss things generally further, please do not hesitate to contact:

Dr Jagdeep Singh
 Clinical Research Fellow
 University of Dundee
 Division of Cardiovascular and Diabetes Medicine
 Medical Research Institute
 Mail Box 2, Ninewells Hospital and Medical School
 Dundee DD1 9SY
 Tel: (01382) 383221
 Email: jxsingh@dundee.ac.uk

Contact Numbers if unwell during the trial

9.2 INFORMED CONSENT FORM



Study Number: (EudraCT) 2014 -2014-002742-42

Participant Identification Number for this trial:

PARTICIPANT CONSENT FORM

Title of Study: **The REFORM Trial** - Research into the Effect Of SGLT2 inhibition on left ventricular Remodelling in patients with heart failure and diabetes Mellitus

Name of Researcher:

Chief Investigator: Professor Chim Lang

Principal Investigator: Dr Jagdeep Singh

Please initial box

1. I confirm that I have read and understand the information sheet dated 25th April 2016 (version 5.0) for the above study. I have had the opportunity to consider the information, to ask questions, and have had them answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by the research team or from the regulatory authorities, NHS Tayside, or the University of Dundee (or their appointed third party), where it is relevant to my taking part in this study. I give permission for these individuals to have access to my records. ☐
4. I agree to my GP being informed of my participation in this study. **YES/NO** (please circle) ☐
5. I understand and agree that the information that I provide will be gifted by myself, and analysed by members of the study team or stored (anonymised) for up to 15 years and can be used for future, as yet unspecified, medical research into health, illness and medical treatment. ☐
6. I agree to use an adequate form of contraception as discussed in the information sheet (if applicable) ☐



7. **OPTIONAL**

I agree to donate an additional 10ml of blood for genetic research purposes and understand that this may be stored indefinitely for future research use.

YES/NO (please circle)

☐

8. **OPTIONAL**

I agree to donate an additional separate 20ml blood sample at the start and end of the study for research purposes and understand that any surplus not used may be stored indefinitely for future research use.

YES/NO (please circle)

☐

9. I agree to take part in the above study.

☐

Name of participant

Date

Signature

Name of person taking consent

Date

Signature

Original to be kept with TMF, 1 copy for participant; 1 copy for hospital notes

9.3 INSULIN SELF MONITORING CHART



REFORM TRIAL GLUCOSE MONITORING SHEET

		EVENING DOSE					MORNING DOSE				
Day		Pre-breakfast Test					Pre-dinner Test				
		<5	5 - 7	7.1 - 11	>11	DOSE	<5	5 - 7	7.1 - 11	>11	DOSE
1											
3											
5											
7											
9											
11											
13											



Reduce insulin by 2 units – **Contact diabetic clinic if occurs twice**



Continue current dose



Increase dose by 2 units



Increase dose by 4 units

9.4 SHORT FORM 36 QUESTIONNAIRE

Patient ID :

Baseline / Final

RAND 36-ITEM HEALTH SURVEY

Date Performed: / /

1. In general, would you say your health is:	
Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

2. Compared to one year ago, how would you rate your health in general now?	
Much better now than one year ago	1
Somewhat better now than one year ago	2
About the same	3
Somewhat worse now than one year ago	4
Much worse now than one year ago	5

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

(Circle One Number on Each Line)

	Yes, Limited a Lot	Yes, Limited a Little	No, Not limited at All
3. Vigorous activities , such as running, lifting heavy objects, participating in strenuous sports	[1]	[2]	[3]
4. Moderate activities , such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	[1]	[2]	[3]
5. Lifting or carrying groceries	[1]	[2]	[3]
6. Climbing several flights of stairs	[1]	[2]	[3]
7. Climbing one flight of stairs	[1]	[2]	[3]
8. Bending, kneeling, or stooping	[1]	[2]	[3]
9. Walking more than a mile	[1]	[2]	[3]
10. Walking several blocks	[1]	[2]	[3]
11. Walking one block	[1]	[2]	[3]
12. Bathing or dressing myself	[1]	[2]	[3]

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

(Circle One Number on Each Line)

	Yes	No
13. Cut down the amount of time you spent on work or other activities	1	2
14. Accomplished less than you would like	1	2
15. Were limited in the kind of work or other activities	1	2
16. Had difficulty performing the work or other activities (for example, it took extra effort)	1	2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

(Circle One Number on Each Line)

	Yes	No
17. Cut down the amount of time you spent on work or other activities	1	2
18. Accomplished less than you would like	1	2
19. Didn't do work or other activities as carefully as usual	1	2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

(Circle One Number)

Not at all 1

Slightly 2

Moderately 3

Quite a bit 4

Extremely 5

21. How much **bodily** pain have you had during the **past 4 weeks**?

(Circle One Number)

None 1

Very mild 2

Mild 3

Moderate 4

Severe 5

Very severe 6

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

(Circle One Number)

Not at all 1

A little bit 2

Moderately 3

Quite a bit 4

Extremely 5

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks . . .

(Circle One Number on Each Line)

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
23. Did you feel full of pep?	1	2	3	4	5	6
24. Have you been a very nervous person?	1	2	3	4	5	6
25. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
26. Have you felt calm and peaceful?	1	2	3	4	5	6
27. Did you have a lot of energy?	1	2	3	4	5	6
28. Have you felt downhearted and blue?	1	2	3	4	5	6

29. Did you feel worn out?	1	2	3	4	5	6
30. Have you been a happy person?	1	2	3	4	5	6
31. Did you feel tired?	1	2	3	4	5	6

32. During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

(Circle One Number)

All of the time 1

Most of the time 2

Some of the time 3

A little of the time 4

None of the time 5

How **TRUE** or **FALSE** is each of the following statements for you.

(Circle One Number on Each Line)

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
33. I seem to get sick a little easier than other people	1	2	3	4	5
34. I am as healthy as anybody I know	1	2	3	4	5
35. I expect my health to get worse	1	2	3	4	5
36. My health is excellent	1	2	3	4	5

Participant Initial's : _____ Date : _____

Interviewers Name : _____ Signature : _____ Date : _____

9.5 MINNESOTA LIVING WITH HEART FAILURE QUESTIONNAIRE

MINNESOTA LIVING WITH HEART FAILURE® QUESTIONNAIRE

The following questions ask how much your heart failure (heart condition) affected your life during the past month (4 weeks). After each question, circle the 0, 1, 2, 3, 4 or 5 to show how much your life was affected. If a question does not apply to you, circle the 0 after that question.

Did your heart failure prevent you from living as you wanted during the past month (4 weeks) by -	No	Very Little				Very Much
1. causing swelling in your ankles or legs?	0	1	2	3	4	5
2. making you sit or lie down to rest during the day?	0	1	2	3	4	5
3. making your walking about or climbing stairs difficult?	0	1	2	3	4	5
4. making your working around the house or yard difficult?	0	1	2	3	4	5
5. making your going places away from home difficult?	0	1	2	3	4	5
6. making your sleeping well at night difficult?	0	1	2	3	4	5
7. making your relating to or doing things with your friends or family difficult?	0	1	2	3	4	5
8. making your working to earn a living difficult?	0	1	2	3	4	5
9. making your recreational pastimes, sports or hobbies difficult?	0	1	2	3	4	5
10. making your sexual activities difficult?	0	1	2	3	4	5
11. making you eat less of the foods you like?	0	1	2	3	4	5
12. making you short of breath?	0	1	2	3	4	5
13. making you tired, fatigued, or low on energy?	0	1	2	3	4	5
14. making you stay in a hospital?	0	1	2	3	4	5
15. costing you money for medical care?	0	1	2	3	4	5
16. giving you side effects from treatments?	0	1	2	3	4	5
17. making you feel you are a burden to your family or friends?	0	1	2	3	4	5
18. making you feel a loss of self-control in your life?	0	1	2	3	4	5
19. making you worry?	0	1	2	3	4	5
20. making it difficult for you to concentrate or remember things?	0	1	2	3	4	5
21. making you feel depressed?	0	1	2	3	4	5

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11/10/04